Antidepressant activity of basil leaves essential oil (Ocimum basilicum) in male balb/c mice

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Depression is the most common mental disorder nowadays. It was triggered many searches to find out traditional plants that has antidepressant activity, one of which is sweet basil. Sweet basil oil that consist of eugenol inspected by in vivo using depression-induced male Balb/c mice and compared its antidepressant activity with fluoxetine HCl. This study was to compare antidepressant activity of sweet basil oil (Ocimum basilicum) and fluoxetine HCl evaluated from immobility time and serotonin level in plasma. An experimental study, in vivo, has been done in animal house and biomolecular laboratory medical faculty of Sriwijaya university during May until July 2015. Samples of 32 male Balb/c mice that divided into 4 groups was adapted for 10 days. Tail suspension test was done for 14 days, then mice blood was taken for checking depression serotonin level. In day 15th until 28th, mice given with sweet basil oil with gradually dose (P1, P2, and P3) and fluoxetine HCl (P4). Tail suspension test was done once and mice blood was taken for checking after therapy serotonin level. Data analyzed with SPSS 16. All male mice are similar in body weight, immobility time at tail suspension test I, and depression serotonin level. By tail suspension test, there is significant difference before and after therapy in group P1, P2, and P3 (p < 0.001), group P4 (p = 0.001). There is no dose suitability between sweet basil oil and fluoxetine HCl based on tail suspension test (p < 0.001). Serotonin level in plasma before and after therapy shows significant difference in all groups (p < 0.001). There is no dose suitability between sweet basil oil and fluoxetine HCl based on serotonin level in plasma (p < 0.001). Antidepressant activity of sweet basil oil and fluoxetine HCl is not similar based on immobility time and serotonin level in plasma.

1. Introduction
Depression is a mental disorder that is common today. Depression affects one in five women and one in ten men at some time throughout his life [1]. Depression is included in mood disorders. Mood disorder is a collection of clinical conditions characterized by loss of feeling of control and subjective experience of severe suffering. Depressed patients feel a loss of energy and interest, feelings of guilt, difficulty concentrating, changes in appetite, and thoughts of death or suicide [2,3] Based on the criteria for Pedoman Penggolongan Diagnosis Gangguan Jiwa III (PPDGJ III), depression is divided into three categories are mild, moderate, and severe depression [4].

Depression can be managed by administering pharmacotherapy and psychotherapy. The main indication for the administration of pharmacotherapy in the form of antidepressants is severe depression. Although the provision of short and specific psychotherapy can provide change, the pharmacotherapy approach has been shown to cause major changes in the treatment process, shorten the course of the disorder, and reduce treatment-related costs [2].
Some antidepressants to choose from are selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), cyclic antidepressants, mixed action agents, selective norepinephrine reuptake inhibitors (SNRIs), monoamine oxidase inhibitors (MAOIs), and alternatives (nontraditional antidepressants) [5,6]. Most clinicians choose either tricyclic or tetracyclic antidepressant drugs or SSRIs as first-line drugs in the treatment of severe depressive disorders. Tricyclic and tetracyclic drugs are often chosen because of the level of clinician satisfaction with the classic drug. In addition, the price of the drug is also cheaper than new drugs because it is available in generic formulations [2].

One neurotransmitter that affects depression is 5-hydroxytryptamine or serotonin. A large number of brain functions are affected by 5-HT, including sleep, cognition, sensory perception, motor activity, temperature regulation, response to pain, appetite, sexual behavior, and hormone secretion [5,6]. 5-HT can be detected in blood plasma and regions brain with the ELISA (Enzyme Linked Immune Sorbent Assay) method [7,8].

Currently there have been many studies to find plants that are efficacious as antidepressants. One of them is basil leaves. Basil is taken from essential oils through a distillation containing various chemicals, one of which is eugenol [9,10]. The efficacy of eugenol one of which is antidepressants [11,12]. Previous studies have stated that eugenol can be used as an antidepressant and works like antidepressants MAOIs [13].

One study showed that *Ocimum basilicum* essential oil at a dose of 2.5 x 10-2 ml / Kg BB had an effect as an antidepressant on Balb / c mice measured from immobility time in the Tail suspension test [14]. Another study showed that essential oil of basil leaves had an antidepressant effect with Forced swim test method [15] while other studies showed that basil extract could act as serotonergic antidepressant to reduce depression in mice exposed to electromagnetic field (EMF) [16]. Other studies have also shown that *Ocimum basilicum* has many therapeutic benefits, one of which is as an antidepressant [17].

To test the effectiveness of eugenol as an antidepressant, a test will be conducted, namely tail suspension test (TST). TST is a method used to evaluate the potential of a drug as an antidepressant. What was assessed from this test was immobility time for the Balb / c mice [18]. This study aims to compare the effectiveness of antidepressants of essential oils of basil leaves (*Ocimum basilicum*) and Fluoxetine HCl in terms of immobility time and serotonin levels in blood plasma.

2. Methods
This research is an in vivo laboratory experimental research with post-test only control group design. Place and time of research performed in the animal house and biomolecular laboratory of FK Unsri for 3 months from May to July 2015. Samples were taken randomly with inclusion criteria: 40 male Balb / c mice aged 2 - 3 months, weighing 20-25 grams. Exclusion criteria: mice with anatomical abnormalities, mice that looked sick / dying, and mice that died before the study.

2.1. Stages of research
Tools and materials
- Essential oils of basil leaves that have been certified are purchased from CV. Eteris Nusantara Jogjakarta.
- Fluoxetine is obtained from PT. Dixa Medica.
- The tools used in the tail suspension test method are mouse mice, sonde, hanging poles / shelves for 60 cm tail suspension tests, stopwatches, and video recorders.
- The tools used in blood sampling are 1 cc syringes, hand gloves, EDTA tubes.
- The tool used in the ELISA method is serotonin ELISA kit.

2.2. Work procedures
In this study there were one group without treatment and four treatment groups (P1, P2, P3, and P4). All groups of mice were placed in a plastic enclosure and adapted to feeding for 10 days. Mice were taken from the storage room to the study area in their cages and allowed to adapt for one hour before the study began. After that, during the first to fourteenth days all treatment groups hung their tail for 5 minutes on a gallows, measured immobility time (after 1 minute of not moving) [19]. From day 15 to 28, without tail hanging, group P1 was given basil leaves essential oil with a dose of
2.5 x 10^{-4} mL / kgBB, group P2 was given basil leaves essential oil at a dose of 2.5 x 10^{-3} mL / kgBB, P3 group was given basil leaves essential oil with a dose of 2.5 x 10^{-2} mL / kgBW, and the P4 group was given Fluoxetine HCl with a dose of 0.0052 mg. On the 29th day, tail suspension tests were carried out on all four groups and immobility time was measured.

Blood sampling was carried out in the group without treatment before tail suspension test, and in the treatment group after the first tail suspension test, after therapy, and after the second tail suspension test. Blood samples will be sent to the laboratory to find out the serotonin levels.

2.3. Data analysis
Homogeneity test was carried out on the group using the Lavene test. After that, the effectiveness and group testing was carried out using paired T Test and unpaired T Test. Then the One Way Anova test was carried out to confirm the results of the T test. The final dose test will be conducted using Post Hoc Test. All analyzes used the SPSS version 16 computer statistical test. Furthermore, dose equivalence was carried out using Simple Linear Regression.

3. Results
This study is an in vivo study which aims to compare the effectiveness of antidepressants of basil leaves essential oil and fluoxetine HCl. Comparison of effectiveness is seen from immobility time on tail suspension test and serotonin levels in blood plasma. The following will be discussed one by one these parameters.

3.1. Tail suspension test
Homogeneity test was done before treatment between groups using Lavene test. P = 0.258, which means there is no difference between groups. Then the normality test was carried out in each group. In the P1, P2, P3, and P4 groups before therapy, normal data distribution results were obtained with p = 0.001 (p <0.05). Homogeneity and normality tests can be seen in table 1.

| Group | Treatment | Mean | Deviation Standard | Maximum | Minimum | P value |
|-------|-----------|------|--------------------|---------|---------|---------|
| P1    | Basil leaves essential oil 2.5 x 10^{-4} ml / kgBW / day | 506,5 | 0,53452 | 506 | 507 | 0,258 |
| P2    | Basil leaves essential oil 2.5 x 10^{-3} ml / kgBW / day | 506,75 | 0,46291 | 506 | 507 |
| P3    | Basil leaves essential oil 2.5 x 10^{-2} ml / kgBW / day | 506,625 | 0,51755 | 506 | 507 |
| P4    | Fluoxetine HCl 0,052 mg/day | 506,5 | 0,53452 | 506 | 507 |

Lavene test (p = 0,05)

After obtaining normal data distribution, paired T tests were carried out in each group. In group P1, there was a difference between before and after therapy with a value of p = 0.001. In group P2, there was a difference between before and after therapy with a value of p = 0,000. In the P3 group, there was a difference between before and after therapy with a value of p = 0,000. In the P4 group, there was a difference between before and after therapy with p = 0,000.

| Group | Treatment | Average immobility time before | Average immobility time after | Paired T tests (p value) |
|-------|-----------|-------------------------------|-------------------------------|-------------------------|
| P1    | Basil leaves essential oil 2.5 x 10^{-4} ml / kgBW / day | 506,5 | 80,25 | < 0,001 |
| P2    | Basil leaves essential oil 2.5 x 10^{-3} ml / kgBW / day | 506,75 | 203,625 | < 0,001 |
| P3    | Basil leaves essential oil 2.5 x 10^{-2} ml / kgBW / day | 506,625 | 403,375 | < 0,001 |
| P4    | Fluoxetine HCl 0,052 mg/day | 506,5 | 502,625 | 0,001 |

Paired T tests (p < 0,05)

After that unpaired T-test is done between groups. There were differences between groups with p = 0,000. Can be seen in table 3.
Table 3. Effectiveness of Basil leaves essential oil compared to Fluoxetine HCl in terms of immobility time between groups.

| Concentration               | Average immobility time after P value |          |
|-----------------------------|--------------------------------------|----------|
| TST II Basil leaves essential oil | 2.5 x 10^4 ml/kgBW/day 80.25 203.625 | < 0.001 |
|                            | 2.5 x 10^3 ml/kgBW/day 80.25 403.375 | < 0.001 |
|                            | 2.5 x 10^2 ml/kgBW/day 203.625 403.375 | < 0.001 |
| TST II Fluoxetine HCl and Basil leaves Essential Oil | 0.195 mg/day 502.625 80.25 | < 0.001 |
|                            | 2.5 x 10^4 ml/kgBW/day 502.625 203.625 | < 0.001 |
|                            | 2.5 x 10^3 ml/kgBW/day 403.375 | < 0.001 |

Unpaired T tests (p < 0.05)

T test results were clarified using the one way ANOVA test. P = 0.000 was obtained. Means that the T test is correct. After that, a dose suitability test is performed using the Post Hoc test. P = 0.000 was obtained.

Table 4. Dose suitability test for Basil leaves essential oils and Fluoxetine HCl in terms of immobility time.

| Variable | P1 | P2 | P3 | P4 |
|----------|----|----|----|----|
| P1       | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| P2       | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| P3       | < 0.001 | < 0.001 | < 0.001 | < 0.001 |
| P4       | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

Post Hoc test (p = 0.05)

3.2. Serotonin levels in blood plasma

Serotonin levels examined in this study were serotonin levels when mice were depressed and after therapy. Serotonin levels when depressed mice were taken after tail suspension test for 14 days. Serotonin levels after therapy is taken after 14 days of therapy are completed. Serotonin levels in blood plasma are examined using ELISA. The ELISA test was conducted in the biomolecular laboratory of FK Unsi Palembang. Table 5 shows serotonin levels in the blood plasma during depression and after therapy.

The homogeneity test of serotonin levels when depressed mice were carried out using lavene test, and p = 0.591 was obtained, meaning that there were no differences between groups. The normality test for each group is carried out. In the P1, P2, P3, and P4 groups before therapy obtained normal data distribution results with p = 0.000 (p <0.05). This shows that all data is normally distributed. Test of homogeneity and normality of serotonin levels in blood plasma when depressed mice can be seen in Table 5.

Table 5. Tests for homogeneity of serotonin levels in blood plasma when mice are depressed.

| Mean Deviation Standard | Maximum | Minimum | Homogeneity |
|------------------------|---------|---------|-------------|
| P1 208.8125 5.98007 | 216.5   | 201.5   | 0.591       |
| P2 207.3725 5.20602 | 215.3   | 201.5   |             |
| P3 208.8125 5.98007 | 216.5   | 201.5   |             |
| P4 210.9625 3.72148 | 215.3   | 204.9   |             |

Lavene test (p < 0.05)
Paired T tests were carried out in each group to assess effectiveness. In the P1, P2, P3, and P4 groups there was a difference between the time of depression and after therapy with \( p = 0.000 \). The effectiveness of basil leaves essential oil and Fluoxetine HCl oil in terms of serotonin levels in blood plasma in groups can be seen in Table 6.

**Table 6. Effectiveness of Basil leaves essential oil and Fluoxetine HCl in terms of serotonin levels in blood plasma in groups.**

| Group | Treatment                          | Average immobility time before | Average immobility time after | Paired T-tests (p-value) |
|-------|-----------------------------------|--------------------------------|-------------------------------|--------------------------|
| P1    | Basil leaves essential oil 2.5 x \( 10^{-4} \) ml/kgBW/day | 208,8125                       | 268,8375                     | \(<0.001\)               |
| P2    | Basil leaves essential oil 2.5 x \( 10^{-3} \) ml/kgBW/day | 207,3625                       | 328,7                        | \(<0.001\)               |
| P3    | Basil leaves essential oil 2.5 x \( 10^{-2} \) ml/kgBW/day | 208,8125                       | 416,025                      | \(<0.001\)               |
| P4    | Fluoxetine HCl 0.052 mg/day       | 208,8735                       | 506,552                      | \(<0.001\)               |

Paired T test (\( P<0.05 \))

The unpaired T test was carried out between groups to compare the effectiveness of antidepressants based on serotonin levels in blood plasma. There were differences between groups with \( p = 0.000 \). The effectiveness of basil leaves essential oil compared to Fluoxetine HCl in terms of serotonin levels in blood plasma between groups can be seen in Table 7.

**Table 7. Effectiveness of basil leaves essential oil compared to Fluoxetine HCL in terms of serotonin levels in blood plasma between groups.**

| Concentration | Average serotonin level in blood plasma after treatment with Basil leaves essential oil | P value |
|---------------|--------------------------------------------------------------------------------------|--------|
| 2.5 x \( 10^{-4} \) ml/kgBW/day | 268,8375 | \(<0.001\) |
| 2.5 x \( 10^{-3} \) ml/kgBW/day | 328,7 | \(<0.001\) |
| 2.5 x \( 10^{-2} \) ml/kgBW/day | 268,8375 | \(<0.001\) |
| 2.5 x \( 10^{-3} \) ml/kgBW/day | 416,025 | \(<0.001\) |

Serotonin level in blood plasma after treatment with Fluoxetine HCl and Basil leaves essential oil:

| Concentration | Average serotonin level in blood plasma after treatment with Basil leaves essential oil | P value |
|---------------|--------------------------------------------------------------------------------------|--------|
| 0.195 mg/day | 506,552 | \(<0.001\) |
| 2.5 x \( 10^{-4} \) ml/kgBW/day | 268,8375 | \(<0.001\) |
| 2.5 x \( 10^{-3} \) ml/kgBW/day | 506,522 | \(<0.001\) |
| 2.5 x \( 10^{-2} \) ml/kgBW/day | 328,7 | \(<0.001\) |

Unpaired T test (\( p<0.05 \))

T test results were clarified using the one way ANOVA test. \( P <0.001 \) was obtained. This shows that the results of the T test are correct. Dose suitability test is done using Post Hoc test. \( P <0.001 \) was obtained.

**Table 8. Dose suitability test.**

| Variable | P1      | P2      | P3      | P4      |
|----------|---------|---------|---------|---------|
| P1       | \(<0.001\) | \(<0.001\) | \(<0.001\) | \(<0.001\) |
| P2       | \(<0.001\) | \(<0.001\) | \(<0.001\) | \(<0.001\) |
| P3       | \(<0.001\) | \(<0.001\) | \(<0.001\) | \(<0.001\) |
| P4       | \(<0.001\) | \(<0.001\) | \(<0.001\) | \(<0.001\) |

*Post Hoc Test (\( p = 0.005 \))
4. Discussion
In this study, the effectiveness of antidepressant essential oils of basil leaves (*Ocimum basilicum*) and Fluoxetine HCl was seen from immobility time with the tail suspension test method and serotonin levels in blood plasma. Judging from immobility time, the four treatment groups show the number of seconds that is not much different at the start of the method and when depression can be seen from the homogeneous data and normal data distribution. However, after being given therapy the four treatment groups showed different numbers.

The difference in immobility time before and after therapy in the group with Fluoxetine HCl therapy can be due to insufficient duration of dosing. According to Harold et al, all classes of antidepressants take three to four weeks to show a meaningful therapeutic effect.

Tao G et al found that eugenol is a monoamine oxidase inhibitor (MAOI). MAOI works to inhibit the enzyme monoamine oxidase which functions to metabolize the neurotransmitters, serotonin and norepinephrine. In the therapy group of essential oils of small doses of basil leaves, there was a difference in immobility time before and after therapy, this could be due to insufficient dosage where only a few MAO enzymes were bound by eugenol so that the metabolism of neurotransmitters (serotonin and norepinephrine) still occurred.

In the treatment of medium-dose basil leaves essential oil, the binding of MAO enzyme occurs more than in the treatment of small doses of basil leaves essential oil. However, there is still a difference in immobility time before and after therapy. However, the antidepressant effect has been seen in this clinical dose.

The difference in immobility time before and after therapy in the group with the therapy of essential oils of large doses basil leaves essential oil can be due to the inadequate dose. The binding of the MAO enzyme can occur more than moderate dose therapy and the antidepressant effect on this dose has been seen, even doubling compared to the moderate dose. However, immobility time is not equal to Fluoxetine HCl, because not all of the MAO enzyme binding.

The antidepressant effects shown in all treatment groups give rise to the possibility that this can occur due to cognitive impairment. To determine cognitive function can be done passive avoidance test, which in this study was not carried out. But it can be seen in the behavior of mice during the second tail suspension test, where the mice refuse and avoid when the tail suspension test will be carried out again. This can indicate that cognitive mice are still good because they still remember the treatment and choose to avoid it.

From the inter-group effectiveness test it can be concluded that the three doses of basil leaves essential oil are not as effective as Fluoxetine HCl in terms of immobility time. This can be because the antidepressant effect caused by the dose of basil leaves essential oil in this study cannot match the antidepressant effect of Fluoxetine HCl. This result is different from previous studies by RR. Lely and Liza A, where the basil leaves essential oil is compared with placebo, not with drug of choice. From the Post Hoc test it can be concluded that in this study the dose of basil leaves essential oil used has not matched the Fluoxetine HCl dose. This happened because immobility time before and after treatment of basil leaves essential oil was significantly different with immobility time before and after Fluoxetine HCl therapy. Therefore there is no dose of conformity.

Looking at the second parameter, the level of serotonin in the blood at the time of depression, the four treatment groups showed numbers that were not much different, characterized by homogeneous data and normal data distribution. But after therapy, all four groups showed different numbers. Based on serotonin levels in the blood, there was an increase after therapy in the P1, P2, P3, and P4 groups. However, an increase in serotonin levels in blood plasma after therapy in groups P1, P2, and P3 did not match the increase in serotonin levels in blood plasma after therapy in the P4 group. This is because the dose of essential oil of basil leaves is less large. In addition, the mechanism of action between Fluoxetine HCl and basil leaves essential oil is different so that the velocity causes different antidepressant effects.

In the small group of basil leaf essential oil therapy group, there has been a small increase in serotonin levels after therapy. This can happen because small doses of essential oils only bind a small portion of the MAO enzyme so that serotonin metabolism still occurs. Likewise on the therapy of essential oils of medium-dose basil leaves. The binding of the MAO enzyme occurs more so serotonin metabolism still occurs. But still seen an increase in serotonin levels after therapy even reached twice that of small doses. In the treatment of basil leaves essential oil with large doses, the binding of the
MAO enzyme is even more but not all, so that the neurotransmitter metabolism still occurs. Increased serotonin levels after therapy have doubled compared to moderate doses.

From unpaired tests showed that multilevel doses of essential oils had significantly different antidepressant efficacy with Fluoxetine HCl. This can be caused by a small dose of essential oil than it should, where the binding of the MAO enzyme still occurs so that serotonin metabolism continues. The results of the Post Hoc test showed that no dose of basil leaves essential oil was found to match the Fluoxetine HCl dose so that the dosage of essential oil that was in accordance with Fluoxetine HCl could not be determined.

5. Conclusion
Basil leaves essential oil has an antidepressant effect with a dose dependent tendency, which means that at small doses it starts to show effect, medium and large doses of the effect increase gradually in terms of immobility time on the tail suspension test and serotonin levels in blood plasma. The effectiveness of antidepressant basil leaves essential oil in large doses is not the same as Fluoxetine HCl in terms of immobility time on tail suspension tests and serotonin levels in blood plasma. There is no suitability for dosage of basil leaves essential oil and Fluoxetine HCl. Therefore, dose equivalence does not need to be done.

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