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The Effect of Kembang Leson Essential Oil on Locomotor Activity of Mice (*Mus Musculus* L.) Balb-C

Nurul Suwartiningsih[1], Raja Ahmad Idham[2] , Ambar Pratiwi[3]
[123]Laboratory of Ecology and Systematics, [3]Laboratory of Biochemistry, [2]Department of Biology, Faculty of Applied Science and Technology, Universitas Ahmad Dahlan,
Jl. Ring Road Selatan, Tamanan, Banguntapan, Bantul Yogyakarta 55166, Indonesia. Tel. +62-274-540971, Fax. +62-274-519739. [1]Email: nurul.suwartiningsih@bio.uad.ac.id

Abstract. Kembang leson essential oil is known to have a relaxing effect so that it can be used as aromatherapy to treat psychological problems. The research was carried out to determine the effect of kembang leson essential oil on locomotor activity of mice (*Mus musculus* L.) Balb-C. The essential oil was isolated with Stahl steam and tested on Balb-C mice with a volume of 0.1 mL, 0.2 mL and 0.5 mL. A paired sample T-test is done to test the average difference between pre-treatment and post-treatment data. ANOVA test is done to test the average difference in post-treatment locomotor activity in each volume. The results show that kembang leson essential oil can increase swimming endurance along with decreasing immobility time and decreasing cage rotation which statistically significant between pre-treatment and post-treatment. The most optimum volume of essential oil influenced locomotor activity is 0.5 mL, with an increase in swimming endurance up to 74.40% and a decrease in the rotary cage up to 50%. Kembang leson essential oil can affect the locomotor activity of Balb-C mice so that it can be developed into pharmaceutical preparations for aromatherapy.

Keywords: antidepressant, essential oil, kembang leson, locomotor activity, aromaterapy.

Running title: kembang leson essential oil

INTRODUCTION

Indonesia has the abundance of plants, including plants that produce essential oils (Sofiani and Rimadani 2017). Essential oil producing plants are estimated to reach 120-200 species and most have the potential to be processed into essential oils (Rusli 2010). This essential oil can be used as a number of aromatherapy products, including to relax the body, food additives, cosmetics, and fragrances that have many benefits (Sofiani and Rimadani 2017). One of the essential oils that are used is kembang leson essential oil. Kembang leson essential oil is widely used as aromatherapy because it is believed to be able to relieve fatigue in the recovery process for patients (Pratiwi and Utami 2018). Kembang leson is a mixture consisting of various flowers and rhizomes whose composition is very diverse such as turmeric (*Curcuma longa* Linn.), ginger (*Zingiber officinale* Rosc.), cloves (*Syzyggium aromaticum* (L.) Merr and Perry), *cumin* (*Cuminum cyminum* L.), nutmeg (*Myristica fragrans* Houtt), various leaves, various flowers, etc. which are commonly found in Central Java and the Special Region of Yogyakarta (DIY). There are various types of essential oil components found in kembang lesons, among which the most commonly found are champhene, benzene methyl cymene, camphor, cyclohexane methanol and curdione (Pratiwi and Utami 2018). The components of essential oils interact quickly when inhaled, stimulating the alfactory system in the central nervous system, then stimulating the nerves in the brain under the equilibrium of the cerebral cortex (Buckle 1999). The content in essential oils has a relaxing effect so it is commonly used as an aromatherapy mixture to treat psychological problems in the form of stress (Rusli 2010).

Stress is a response from the body caused by the body's ability to face loads beyond its ability and has a nonspecific nature. Depression caused by negative stress or distress will statistically decrease the amount of movement actin of experimental animals compared to the control. Movement activity caused by changes in electrical activity through the central nervous system due to changes in the permebiality of the post-synapnatic cell membrane and the release the transmitter, is called locomotor activity (Gilman *cit* Muchtaridi 2015).

Locomotor activity in experimental mice (*Mus musculus* L.) can be seen based on the number of movements of mice when turning the cage (Muchtaridi 2015). Physical endurance activity can be seen in swimming resistance in experimental mice which is carried out after 30 minutes of giving the test material orally (Deswati dan Maryati 2107). The results of Muchtaridi (2015) study showed that seven aromatic plants from Indonesia include basil (*Ocimum basilicum* L.), eucalyptus leaves (*Melaleuca leucadendra* L.), nutmeg, kilemo bark (*Litsea cubeba*. Lour.), herbaceous lemongrass (*Cymbopogon citrates* L.), gowah rhizome (*Alpinia malaccensis* Roxb.), and cananga flower (*Cananga odorata* Lam.) can affect locomotor activity, especially nutmeg essential oil which has a greater effect in reducing locomotor activity in experimental mice. Research that examines the relationship between kembang leson essential oil and locomotor activity in experimental mice has not been carried out. So it is necessary to do research on the effect of kembang leson essential oil on locomotor activity in experimental mice.

MATERIALS AND METHODS

This research was conducted at the Laboratorium Pengamatan Hama Penyakit Tanaman (LPHPT) Bantul for the distillation of kembang leson essential oil and Laboratory of Animal Structure and Physiology Ahmad Dahlan University for the treatment of tested animals.

Procedures

Distilation of essential oil

Fresh kembang lesons was weighed as much as 6 kg then cut into pieces, distilled with a Stahl steam distillator for 6 hours with 100-105 ℃ boiling point.

Preparation of test animal

This study used experimental mice Balb-C strain 6 weeks old weighing 15-25 grams as many as 30 individuals. The mice were acclimatized by being placed in a plastic cage with a size of 80 x 40 x 25 cm for seven days, lighting 12 hours of light and 12 hours of darkness, feed according to standard (ad libitium), the cage is kept clean, and is not noisy. The experimental design was a completely randomized design (CRD) which consisted of four groups: control (K-), received treatment of kembang leson essential oil with a volume of 0.1 ml (P1), volume of 0.3 ml (P2), and volume of 0.5 ml (P3). Each treatment group used six mice then divided into two groups, three to be tested for swimming endurance and three to be tested for rotary cage endurance.

Predator exposure test

The acclimatized mice were exposed to predators by being brought closer to the cats for 60 minutes/day in each cage for seven days, from day 8 to day 14. This was done to give stress to the mice.

Pre-treatment of swimming endurance

Mice were tested for locomotor activity in the form of swimming endurance as a pre-treatment (pretest) which was carried out on day 15. Mice that were tested for swimming endurance were put in a 12 liters bucket filled with water. Swimming endurance was measured from the time the mice started swimming until drowning, marked by the time the mice were under the water surface for 5 seconds. The time of endurance in the test group was compared with the control group.

Pre-treatment of rotary cage

Mice were tested for locomotor activity in the form of the ability to rotate the rotary cage as a pre-treatment (pretest) which was carried out on day 15. The mice tested for the rotary cage were placed in the rotary cage. The number of rotations in the rotary cage was recorded for 15 minutes starting after the mice turned the rotary cage. The number of test groups was compared with the control group.

Essential oil treatment

The treatment of kembang leson essential oil in mice was carried out using an inhalator with a size of 40 x 20 x 30 cm which functions to inhalation of kembang leson essential oil in mice with volume variations of 0.1 ml, 0.3 ml and 0.5 ml for 10 minutes/day for seven day on day 16 to the day 22.

Post-treatment of swimming endurance and rotary cage

Done with the same procedure as the pre-treatment (pretest). Data were taken on day 23.

Data analysis

A paired sample T-test is done to test the average difference between pre-treatment and post-treatment data. ANOVA test is done to test the average difference in post-treatment locomotor activity in each volume.

RESULTS AND DISCUSSION

This research uses kembang leson which is obtained from Gamping Market, Sleman, Yogyakarta Special Region. Kembang lesons used consist of various flowers and rhizomes including ginger (*Curcuma zanthorrhiza* Roxb.), temu giring (*Curcuma heyneana* Val.), temu ireng (*Curcuma aeruginosa* Roxb.), dlingo (*Acorus calamus* L.), bay leaves (*Syzygium polyanthum* (Wight) Walpers), lemongrass leaves (*Cymbopogon citrates* L.), pandan leaves (*Pandanus amaryllifolius* Roxb.), lime leaves *(Citrus hystrix* D.C), cananga flowers (*Cananga odorata* Lam.), roses (*Rosa santana* L.) and magnolia flowers (*Magnolia x alba* D.C). Extraction was carried out by Stahl water distillation. The extract of the essential oil was obtained as much as 15.60 ml. The results showed the increase of swimming endurance duration from pre-treatment to post-treatment of all the test groups (P1, P2 and P3) except in control treatment (K-) (Table 1).

Table 1. Pre-treatment and post-treatment swimming endurance duration.

No	Treatment	Swimming Endurance (seconds)				Increased Swimming Endurance
		Pre-treatment		Post-treatment		
		Average	Standard deviation	Average	Standard deviation	
1.	K-	221	17.00	221	15.62	0.00%
2.	P1	161	11.59	233	8.71	41.14%
3.	P2	175	18.52	264	8.00	50.86%
4.	P3	168	5.29	293	4.04	74.40%

It can be seen on Table 1 that the highest increase in the duration of swimming endurance is the group of depressed mice that received treatment of kembang leson essential oil with a volume of 0.5 ml (P3) with an increase of 74.40%. The lowest increase in the duration of swimming endurance was the normal group of mice that did not get depressed and did not receive treatment (K-) with an increase of 0%. The comparison of the duration of pre-treatment and post-treatment swimming endurance can be seen in Figure 1.

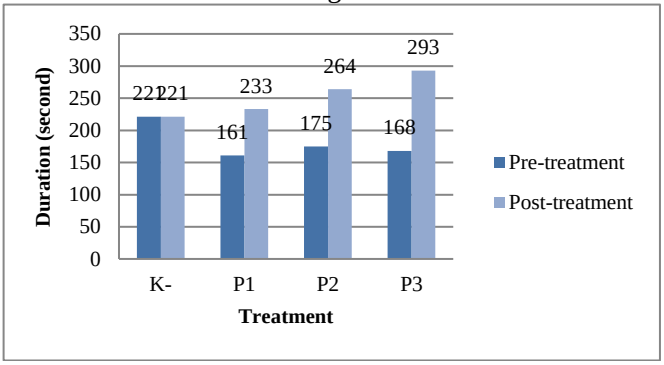

Figure 1. Pre-treatment and post-treatment swimming endurance duration comparison diagram.

The results in Figure 1 indicate that there was an increase in the duration of swimming endurance in all test groups (P1, P2 and P3) except for the control (K-) after being given treatment (post-treatment). This also shows that the higher the volume of kembang leson essential oil, the higher swimming endurance of the mice, except for the control (K-). The data duration of swimming endurance were then tested using the paired sample T-test to determine the significant difference in swimming endurance duration before and after treatment. Result of the paired sample T-test summarized in Table 2.

Table 2. Results of paired sample T-test on swimming endurance duration

Treatment	Significant value	Conclusion
K-	0.950	Not significant
P1	0.022	significant
P2	0.026	significant
P3	0.002	significant

Table 2 showed that there is a significant difference in the duration of swimming endurance before being given kembang lesons (pre-treatment) and after being given kembang lesons (post-treatment) in all the test groups (P1, P2 and P3) except for the control (K-). Post-treatment swimming endurance duration data were also compared using the ANOVA test. Anova test results showed the value of $p = 0.000$ ($p < 0.05$) is obtained, which indicates that there is a significant difference of post-treatment swimming endurance duration in each treatment. Furthermore, the Least Significant Difference (LSD) test was carried out to determine which groups showed significant differences (Table 3).

Table 3. The results of the Least Significant Difference (LSD) test between post-treatment groups of swimming endurance duration

Treatment	Average of swimming endurance duration in post-treatment ($\overline{X}$)
P3	79[a]
P2	91[a]
K-	115[b]
P1	116[b]

Table 3 showed that there is no significant difference between control (K-) and 0.1 ml volume (P1). Meanwhile, negative control (K-) and volume 0.1 ml (P1) were significantly different from volume 0.3 ml (P2) and volume 0.5 ml (P3). The volume of 0.5 ml (P3) was not significantly different from the volume of 0.3 ml (P2). This shows that the volumes of 0.3 ml (P2) and 0.5 ml (P3) are the optimum levels which can affect the ability of Balb-C mice for swimming endurance. The comparison of pre-treatment and post-treatment rotary cage endurance can be seen in Table 4.

Table 4. Pre-Treatment and Post-Treatment Rotary Cage Endurance

No	Treatment	Quantity of Cage Rotation				Decreased Rotary Cage
		Pre-treatment		Post-treatment		
		Average	Standard deviation	Average	Standard deviation	
1.	K-	116	9,29	115	12,12	0.86%
2.	P1	138	5	116	3,51	15.94%
3.	P2	147	7,21	91	6,24	38.10%
4.	P3	158	9,01	79	2,51	50%

Table 4 showed that the the highest decrese in the endurance of rotary cage was the group of depressed mice that received treatment of kembang flower essential oil with volume of 0.5 ml (P3) with a decrease percentage of 50%. The lowest decrease in the endurance of the rotary cage was the group of normal mice that did not get depressed and did not receive treatment (K-) with a decrease percentage of of 0.86%. The comparison of the pre-treatment and post-treatment rotary cage endurance can be seen in Figure 2.

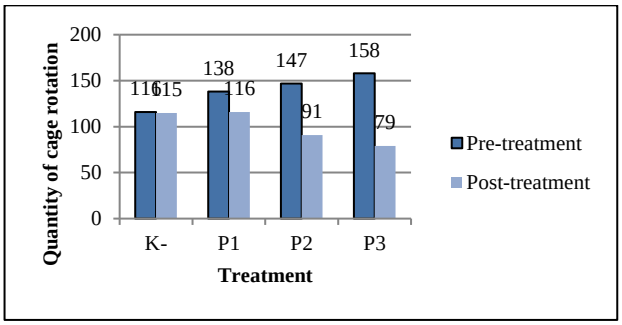

Figure 2. Pre-treatment and post-treatment rotary cage endurance comparison diagram.

The results showed in Figure 2 indicated that there was a decrease in the endurance of the rotary cage in all test groups (P1, P2 and P3) except for the control (K-) after being given treatment (post-treatment). This also showed that the higher the volume of the kembang leson essential oil, the lower the endurance of the rotary cage. The data of rotary cage endurance were then tested using the paired sample T-test to determine the significant difference in rotary cage endurance before and after treatment. Result of the paired sample T-test summarized in Table 5.

Table 5. Results of paired sample T-test on rotary cage endurance

Treatment	Significant value	Conclusion
K-	0.370	Not significant
P1	0.015	significant
P2	0.001	significant
P3	0.002	significant

Table 5 showed that there is a significant difference in the quantity of rotary cage endurance before being given kembang lesons (pre-treatment) and after being given kembang lesons (post-treatment) in all the test groups (P1, P2 and P3) except for the control (K-). Post-treatment rotary cage endurance duration data were also compared using the ANOVA test. Anova test results showed the value of p=0.000 ($p < 0.05$) is obtained, which indicates that there is a significant difference of post-treatment rotary cage endurance duration in each treatment. Furthermore, the Least Significant Difference (LSD) test was carried out to determine which groups showed significant differences (Table 6).

Table 6. The results of the Least Significant Difference (LSD) test between post-treatment groups of rotary cage endurance

Treatment	Average of rotary cage endurance quantity in post-treatment ($\overline{X}$)
K-	115^{a}
P1	116^{a}
P2	91^{b}
P3	79^{b}

Table 6 showed that there is no significant difference between negative control (K-) and 0.1 ml volume (P1). Meanwhile, negative control (K-) and volume 0.1 ml (P1) were significantly different from volume 0.3 ml (P2) and volume 0.5 ml (P3). The volume of 0.5 ml (P3) was significantly different from the volume of 0.3 ml (P2). This indicated that the volume of 0.5 ml (P3) is the optimum level which can affect the ability of Balb-C mice to rotate the cage.

Discussion

There is a significant difference in the duration of swimming endurance before being given kembang lesons (pre-treatment) and after being given kembang lesons (post-treatment) in all the test groups (P1, P2 and P3) except for the control (K-) (Table 2). It can be said that giving kembang leson extract can increase the duration of swimming endurance or decrease the immobility time. This is reinforced by previous research conducted by Puspitasari (2016), which states that aromatherapy can increase swimming resistance along with a decrease in immobility time. The results of this study could be influenced by the active compounds contained in kembang leson essential oil. According to Pratiwi and Utami (2018), there are various types of essential oil components including the most commonly found champhene, benzene methyl cymene, camphor, cyclohexana methanol and curdione. The compounds contained in these essential oils are believed to have a relaxing effect, where these compounds when inhaled stimulate the alfactory system in the central nervous system, then stimulate the nerves in the brain under the equilibrium of the cerebral cortex (Buckle, 1999) and stimulate movement activity (Gilman *cit* Muchtaridi 2015).

The higher the volume of kembang leson essential oil, the higher swimming resistance of the mice, except for the control (K-) (Figure 1). The highest increase in the duration of swimming endurance is the group of depressed mice that received treatment of kembang leson essential oil with a volume of 0.5 ml (P3) with an increase of 74.40% (Table 1) and it was significant statistically (Table 3). The content of metabolites contained in the essential oil of kembang leson has an antidepressant effect that can affect locomotor activity. According to Puspitasari (2016), increased physical activity in depressed conditions can also be accompanied by an improvement in mood experienced and a psychological improvement with a decrease in cortisol levels in test animals. This improvement resulted in an increase in activity in test animals, where the test animals tended to move more actively in the water to save themselves and did not stay long in a state of rest/despair during the forceswimming test. The

components of the essential oil compounds used in this study contain compounds with a relaxing effect, in which the more volatile oil compounds that are inhaled allow the test animals to relax more quickly so that they can increase physical activity in the form of swimming endurance.

There is a significant difference in the quantity of rotary cage endurance before being given kembang lesons (pre-treatment) and after being given kembang lesons (post-treatment) in all the test groups (P1, P2 and P3) except for the control (K-) (Table 5). It can be said that the kembang leson can reduce the endurance of the rotary cage. These results are in accordance with previous research conducted by Muchtaridi (2015), which concluded that giving a substance in the form of aromatherapy will reduce the number of cage rotation performed by experimental animals. This can be influenced by the active compounds contained in the essential oil of the kembang leson. According to Pratiwi and Utami (2018), there are various types of essential oil components, including the most commonly found champhene, benzene methyl cymene, camphor, cyclohexana methanol, and curdione. The compounds contained in these essential oils are believed to have a relaxing effect, where the compound is activated when inhaled, the compound will stimulate the alfactory system in the central nervous system, then this system will stimulate the nerves in the brain under the equilibrium of the cerebral cortex (Buckle 1999) and reducing movement activity (Gilman *cit* Muchtaridi, 2015) resulting in decreased locomotor activity in rotary cage.

The higher the volume of the kembang leson essential oil, the lower the endurance of the rotary cage (Figure 2). The highest decrese in the endurance of rotary cage was the group of depressed mice that received treatment of kembang flower essential oil with volume of 0.5 ml (P3) with a decrease percentage of 50% (Table 4) and it was significant statistically (Table 6). Efruan et al. (2016) states that the components of essential oils provide benefits to reducing the locomotor activity of mice in the presence of the main compound, namely 1,8-cineol and several other dominant compounds such as citronellol, citronellal and a-terpineol. Buchbauer et al. (1991), also said that compounds that smell good or fragrance of the essential oils of a plant material are also proven to affect locomotor activity. This is cuased the more essential oil compounds that are inhaled allow the test animals to relax more quickly and even experience sedatives, thus allowing a decrease in locomotor activity in the test animals in the form of a rotary cage more quickly.

CONCLUSIONS

Based on the research that has been done we can conclude that the effect of kembang leson essential oil on the locomotor activity of experimental mice, can increase locomotor activity in the form of swimming endurance and reduce locomotor activity in the form of a rotary cage. The most optimum volume of essential oil influenced locomotor activity is 0.5 mL, with an increase in swimming endurance up to 74.40% and a decrease in the rotary cage up to 50%.

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