Effect of 10% Lavender Essential Oil Balm on Serum Cortisol Levels in Male Wistar Rats

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Abstract: Lavender (Lavandula angustifolia) is a Mediterranean plant that has developed throughout the world and often used as aromatherapy for relaxation. Balm is one of the topical drug forms that effective for patients with very dry skin and has a higher potency and greater drug penetration. Therefore, this study was conducted to determine the effect of 10% lavender essential oil balm on serum cortisol levels. This study used 36 male Wistar rats divided into 4 groups (negative control = no stressor; positive control = stressor only; placebo = stressor + placebo; L1 = stressor + Lavender 10%), the forced swim test was given as the stressor. Serum cortisol levels were analysed using the Kruskal-Wallis Test (p<0.05) and continued with the Mann-Whitney Test (p<0.05). The result of the serum cortisol levels analysis showed that 10% lavender essential oil balm significantly (p=0.007 and p=0.041) decreased the serum cortisol levels in rats compared to negative control group and positive control group (684.19 ± 54.081 (L1), 712.95 ± 129.589 (C-), and 728.13 ± 48.125 (C+)). These results indicate that lavender essential oil balm can be used as an alternative treatment to relieve stress but should be further researched for other biochemical parameters.

1 INTRODUCTION

The incidence of stress is still high in various groups and professions in the world. According to the World Health Organization, more than 300 million people in 2015 suffer from depression and disability in the world (World Health Organization, 2017). In 2013, the Indonesian Ministry of Health published Basic Health Research (Risksdas) stated that 6% of the total population in Indonesia experienced emotional mental disorder (Kementerian Kesehatan, 2013). Risksdas Data in 2018 showed that the figure had increased to 9.8% (Kementerian Kesehatan, 2018). Untreated stress can lead to various problems. According to a study conducted by Wada et al. (2013) stated that high occupational stress exposure could lead to the onset of depression (Wada et al., 2013). Furthermore, patients diagnosed with acute stress reactions had a greater rate for completed suicide (Gradus et al., 2010). Stress is a stimulus that evokes the release of ACTH and adrenal glucocorticoid (Fink, 2016). Stress causes activation of various physiological responses especially in the endocrine system, the nervous system, and the immune system (Contrada and Baum, 2011). The biological response to stress is differentiated between acute reaction and chronic reaction. Acute reaction triggers a rapid release of noradrenaline and adrenaline through the sympathetic-medullary-adrenal axis (SMA Axis). While the chronic reaction activating the hypothalamic-pituitary-adrenal (HPA Axis) produces cortisol (Matteri et al., 2000).

Cortisol is a glucocorticoid hormone produced by the adrenal glands and synthesized from cholesterol (Silverthorn, 2007). Cortisol levels are regulated by adrenocorticotropic hormone (ACTH), which response to corticotropin-releasing hormone (CRH). Cortisol has widespread action such as reduces inflammation, suppresses the immune system, helps the body to manage stress, and increases blood sugar through gluconeogenesis (Guyton and Hall, 2016). Serum cortisol levels is often used as an indicator of stress conditions (Möstl and Palme, 2002). Forced swim test can be used as a stressor which stimulate the release of cortisol hormone (Khaleel Jameel et al., 2014).

Essential oils are a volatile product of a plant that
has a scent so it is often used for cosmetic products, perfumes, and aromatherapy. Essential oils obtained from plants by steam distillation. One of the essential oils that can be used is lavender. Lavender has more than 30 species, dozens of subspecies, and hundreds of hybrids, some of which are Lavandula angustifolia (English Lavender), Lavandula stoechas (French Lavender), Lavandula latifolia, and Lavandula intermedia (Cavanagh and Wilkinson, 2002).

Lavandula angustifolia derived from the Lamiaceae family is a native Mediterranean plant and thrives in highland areas (Verma et al., 2010). Lavender has two major constituents, there are linalool and linalyl acetate (Kivrak, 2018). Lavender essential oil has many benefits such as relieve pain, improve sleep quality, antimicrobial, anxiolytic, and repellent (Cavanagh and Wilkinson, 2002).

The administration of lavender essential oil can be oral, inhalation, and topical (Dornic et al., 2016). Research showed the use of lavender essential oil in inhalation can decrease the cortisol levels in blood and saliva (Kim et al., 2012; Hosseini et al., 2016). Moreover, oral administration of lavender essential oil has an anxiolytic effect (Kasper et al., 2010). The study of Jager et al. (1992) suggested that linalool and linalyl acetate (active components of lavender essential oil) are rapidly absorbed through the skin and reaching peak levels after 19 minutes (Jager et al., 1992).

This study aims to research the usage of natural topical medication like lavender essential oil balm to investigate the effectiveness for stress conditions. Currently, treatment for stress conditions always oriented to synthetic drugs such as psychotropic drugs. These drugs are susceptible to misuse. Serum cortisol levels are the indicator of stress conditions.

2 METHODS

2.1 Animals

Male Wistar rats (2-3 months, 100-200 grams) were habituated for 7 days before the experiment. Foods were given 60 grams and water was available ad libitum. Animals were individually housed in the cage.

2.2 Materials

Placebo balm consists of 1-gram beeswax and 5 ml (4.62 gram) virgin coconut oil as vehicle formula and no active ingredient formula was given. 10% lavender essential oil balm consists of 1-gram beeswax and 5 ml (4.62 gram) virgin coconut oil as vehicle formula and 0.625-gram lavender essential oil (Lavandula angustifolia) as active formula.

2.3 Experimental Procedures

This study used 36 male Wistar rats divided into 4 groups (negative control = no stressor; positive control = stressor only; placebo = stressor + placebo; L1 = stressor + Lavender 10%), the forced swim test was given as the stressor. The cylinder was filled with lukewarm water to a height of 30 cm. Animal was placed in a water-filled cylinder for 10 second every day for 30 days. Lavender essential oil balm was given to the treatment group (L1) on the back (was shaved 2x2 cm) within 30 minutes after the forced swim test. These experiments were conducted between 6:00 AM and 8:00 AM.

All of the rats were sacrificed on the 30th day. The blood was collected via intracardiac puncture and was centrifuged for plasma collection. All plasma was stored in the freezer until assayed by ELISA.

2.4 Statistic

This study was an experimental study with a post-test only control group design. All the results are presented as means ± standard error of the mean. Statistical significance was analysed using the Kruskal-Wallis test with the Mann-Whitney U as post-hoc analysis by SPSS 25.0 software. P<0.05 was considered to indicate a statistically significant difference.

3 RESULT

Mann-Whitney U test showed that 10% lavender essential oil balm significantly decreased the serum cortisol levels in male Wistar rats compared to the negative control group. Mean ± SD of serum cortisol levels in 10% lavender essential oil balm and negative control group were 684.19 ± 54.081 and 712.95 ± 129.589 (Table 1).

Also 10% lavender essential oil balm significantly decreased the serum cortisol levels in male Wistar rats compared to untreated rats. Mean ± SD of serum cortisol levels in 10% lavender essential oil balm and positive control group were 684.19 ± 54.081 and 728.13 ± 48.125 (Table 2).

Results showed no significant difference in
serum cortisol levels in the placebo balm group and positive control group. Mean ± SD of serum cortisol levels in the placebo balm group and positive control group were 699.59 ± 64.135 and 728.13 ± 48.125 (Table 3).

Table 1: Comparison of negative control group and treatment group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C° (mean ± SD)</th>
<th>L° (mean ± SD)</th>
<th>Sig (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cortisol</td>
<td>712.95 ± 129.589</td>
<td>684.19 ± 54.081</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

C°: no stressor and no treatment were given
L°: stressor and 10% lavender essential oil balm were given

Table 2: Comparison of positive control group and treatment group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C° (mean ± SD)</th>
<th>L° (mean ± SD)</th>
<th>Sig (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cortisol</td>
<td>728.13 ± 48.125</td>
<td>684.19 ± 54.081</td>
<td>0.041*</td>
</tr>
</tbody>
</table>

C°: stressor and no treatment were given
L°: stressor and 10% lavender essential oil balm were given

Table 3: Comparison of positive control group and placebo group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C° (mean ± SD)</th>
<th>L° (mean ± SD)</th>
<th>Sig (p&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cortisol</td>
<td>728.13 ± 48.125</td>
<td>699.59 ± 64.135</td>
<td>0.07</td>
</tr>
</tbody>
</table>

C°: stressor and no treatment were given
P: stressor and placebo balm were given

Table 4: Serum cortisol levels in

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Serum cortisol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>9</td>
<td>712.95 ±</td>
</tr>
<tr>
<td>Positive control</td>
<td>9</td>
<td>728.13 ±</td>
</tr>
<tr>
<td>Placebo</td>
<td>9</td>
<td>699.59 ±</td>
</tr>
<tr>
<td>Lavender 10%</td>
<td>9</td>
<td>684.19 ±</td>
</tr>
</tbody>
</table>

4 DISCUSSION

The present study was planned to evaluate the effect of 10% lavender essential oil balm on serum cortisol levels of forced swim test models inducing stress in male Wistar rats. As shown in Table 1 and Table 2, serum cortisol levels were significantly lower after given 10% lavender essential oil balm. These results were similar to the previous study.

Lee and Cho (2014) studied that both lavender essential oil inhalation and rosemary essential oil inhalation reduced saliva cortisol levels in twenty healthy South Korean students. However, the lavender group reduced the saliva cortisol levels more than the rosemary group. Furthermore, lavender essential oil also significantly decreased stress index and mood index in subjects (Lee and Cho, 2014). In another study, four weeks of inhalation of lavender essential oil could reduce the level of saliva cortisol and daytime blood pressure in hypertensive and hypertensive subjects, (Kim et al., 2012). Hosseini et al. (2016) also reported that inhalation of lavender essential oil showed anxiolytic effect that decreased serum cortisol levels in candidates for open-heart surgery (Hosseini et al., 2016).

In this study, the researcher also examined the effects of placebo balm against the serum cortisol levels in male Wistar rats. We showed that no significant differences were observed in serum cortisol levels between the placebo balm and the control group (Table 3). These results indicated that the constituent of placebo balm did not reduce serum cortisol levels.

Lavender essential oil has two major constituents: linalool and linalyl acetate (Verna et al., 2010; Kvrak, 2018). A study was done by Umezu et al. (2006) observed that male mice given lavender essential oil intraperitoneally displayed an anti-conflict effect in the Geller test and Vogel test. Furthermore, the major constituents of lavender essential oil (linalool and linalyl acetate) were also evaluated and the only linalool showed anxiolytic effect (Umezu et al., 2006). Souto-Maior et al. (2011) reported an anxiolytic effect for inhaled linalool on male mice using the elevated plus-maze test and light/dark box test. Also, inhaled linalool did not appear to cause muscle relaxation or motor coordination because no significant decreased time spent on the rotarod test (Souto-Maior et al., 2011). However, Takahashi et al. (2011) concluded that linalyl acetate and linalool act synergistically to induce anxiolytic effect in the elevated plus-maze test (Takahashi et al., 2011).

The mechanism for serum cortisol levels reduction by 10% lavender essential oil balm remains to be determined. These effects may occur through GABA_A receptor modulation by linalool which increases brain GABA levels (Milanos et al., 2017). GABA is the major inhibitory neurotransmission in the brain. GABA suppresses
the activity of the HPA Axis by inhibiting paraventricular nuclei in the hypothalamus (Cullinan et al., 2008). Therefore, impaired secretion of corticotropin-releasing hormone (CRH) by paraventricular nuclei will disturb the secretion of adrenocorticotropic hormone by the anterior pituitary gland (Hannibal and Bishop, 2014). As a result of HPA Axis suppression, the cortisol levels secretion by the adrenal gland will be reduced.

5 CONCLUSIONS

This study was performed to investigate the effectiveness of 10% lavender essential oil balm for stress conditions. These result demonstrate that 10% lavender essential oil balm reduced the serum cortisol levels in male Wistar rats. The 10% lavender essential oil balm determined to have a meaningful anxiolytic effect. These results indicate that lavender essential oil balm can be used as an alternative treatment to relieve stress but should be further researched for other biochemical parameters.

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