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INVOLVEMENT OF MONOAMINERGIC SYSTEM IN THE ANTIDEPRESSANT-LIKE EFFECT OF *Ocimum sanctum* (LINN.) LEAVES EXTRACT IN TAIL SUSPENSION TEST IN MICE

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ABSTRACT

Depression is a psychiatric disorder characterized by decreased mood and affects daily activities. The development of herbal ingredients as complementary therapy becomes increasing in line with the number of patients who avoid side effects in conventional treatment. The purpose of this study is to investigate the potential effects of ethanolic extract of *Ocimum sanctum* L. as an antidepressant as well as evaluate the potential mechanism of action in the monoaminergic system in *in vivo* study. For evaluation of mechanisms of action, mice were treated serotonin synthesis inhibitors (pCPA), non-selective dopamine antagonists (Haloperidol) and selective antagonists D2 dopamine (Sulpiride) as pre-treatment. The *Tail Suspension Test (TST)* method is used as a standardized test that has generally been used to evaluate the antidepressant effects of a drug compound. The results showed that the ethanolic extract of *Ocimum sanctum* L. has antidepressant effect at the dose of 150 mg/kg. Ethanol extract of *Ocimum sanctum* L. was able to decrease the immobility time in TST mice model significantly when given *pre-treatment* with pCPA ($p<0.05$) and haloperidol ($p<0.05$), while sulpiride ($p<0.05$) inhibits the antidepressant effects of ethanolic extract of *Ocimum sanctum* L. significantly. These results showed that the monoaminergic system especially serotonergic and dopaminergic were involved in the antidepressant-like effects of ethanolic extract of *Ocimum sanctum* L. by involving dopamine receptors and has a role in serotonin synthesis.

Keywords: *Ocimum sanctum*, antidepressant, tail suspension test, monoaminegic

BACKGROUND

Depression is a phychiatric disorder that affecting mood and behavior in human. More than 264 million people in the world were affected by this condition (WHO, 2019). According to Riskesdas in 2018, the prevalence of depression

was showed 6,1% in Indonesia, while the prevalence in East Java was 4.5% (Riskedas, 2018). Several drugs including selective serotonin reuptake inhibitors (SSRIs), serotonin & norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) were approved as a standard pharmacological treatment of depression (Katzung, 2012). However, about only 50% patients provide complete remission and produces more side effects after taking these medications. Therefore, the research to find alternatives or complementary drugs to treat depression and also provide more minimal side effects are needed, especially from traditional herbs.

Ocimum sanctum L, which called as Kemangi, is one of Indonesian herbs that has potential as antidepressant. A study showed that the leaves extract of *Ocimum sanctum* L. produced significant antidepressant effect in animal model of depression (Chatterjee, 2011). Eugenol and apigenin were active substance that thought to be responsible mediating this effect (Bhattacharyya & Bishayee, 2013). Eugenol compounds exhibit antidepressant activity by molecular docking (Tao et al., 2005) while Apigenin content also showed antidepressant effects by significantly reducing the duration of immobility using the Forced Swimming Test (FST) method (Guan, 2016). However, the mechanism of action of Ocimum sanctum L. as antidepressant is not fully established.

According to monoamine theory, depressive symptoms are caused by functional deficiencies of monoamine neurotransmitters, such as serotonin (5-HT), dopamine (DA) and norepinephrine (NE) in the central nervous system (Cito et al., 2014). Serotonergic system involves the synthesis of serotonin from tryptophan as a precursor catalyze by the enzyme hydroxylase tryptophan which produces 5-hydroxytriptofan (5-HT) (Herawati, 2008). Decreased synthesis of serotonin is one of the factors that contribute on decreasing the activity of serotonorgic system. Furthermore, decreased neurotransmission of the dopaminergic system in the central nervous system causes physiological changes by reducing the transmission

of DA signals (Dunlop, 2007). Additionally, by blocking the dopamine receptors was also reduced the dopaminergic activity which leads to cognitive, psychomotor and physiological impairments.

This study was aimed to investigate the possible mechanism of action in the antidepressant-like effect of the ethanolic extract of *Ocimum sanctum* leaves (OCE). The involvement of dopamine receptors interaction and serotonin synthesis as the potential mechanism of action were evaluated by *in vivo* study using Tail Suspension Test method as the animal model of depression.

METHODS

Materials

The ethanolic extract of *Ocimum sanctum* L. (OCE) was purchased from Materia Medika Indonesia (Malang, East Java). The preparation of the extract was made using maceration method with ethanol 70% (v/v) as the solvent. Sulpiride (Dogmatil®) and Haloperidol was purchased from Apotek UBAYA with official permission for research experiments. pCPA was purchased from Sigma, Aldrich.

Animals

Male *Balb-c* mice (25-30 g) were used as experimental animal which kept in a 12:12-hour light-dark cycle. All experiments were carried out at temperatures 25±2°C and relative humidity 75±5% with access to food and water *ad libitum*. The habituation phase was carried out for seven days before the tail suspension test. The experiment was carried out in the light phase between 9.00 a.m – 15.00 p.m. The number of animals per group was calculated using the Federer formula. All animal procedures were carried out following guidelines for the Use of Laboratory Animals and UBAYA Research Ethics Commission for animal as experimental subject.

Tail Suspension Test (TST)

Tail suspension test is one of the standard methods to evaluate antidepressant effect of potential substance for depressive treatment. The principle of this method is to hang the animal's tail vertically on a suspension box with adhesive tape of the animal's tail for 6 minutes as shown in Figure 1. The observed

parameter is the duration in seconds (sec) when the animal does not move or does not attempt to escape which refers as immobility time. The duration of immobility time was recorded at the last 5 minutes of observation.

Figure 1. Tail suspension test apparatus.

Determination of effective dose of antidepressant-like effect of OCE

In this evaluation, mice were divided into 4 groups (n = 5 each group): Control group received vehicle (CMC Na, p.o), OCE groups were divided into 3 groups which received the Ocimum sanctum leave extract by per oral (p.o) at the dose of 50 (II), 100 (III), and 150 (IV) mg/kg of body weight. Thirty minutes after the treatment, mice were hanged in the TST apparatus for 6 minutes. Immobility time (s) was recorded at the last 5 minutes of observation.

Determination of the involvement of monoaminergic systems in the antidepressant-like effect of OCE

In this test, mice were divided into 8 groups (n = 6 each group): Vehicle (CMC Na (i.p) + vehicle (CMC Na (p.o) as control group; Vehicle (CMC Na, i.p) + OCE (150 mg/kg, p.o) as OCE group; para-Chlorophenylalanine (100 mg/kg, i.p) + Vehicle (CMC Na, p.o) as pCPA group; Supiride (50 mg/kg, i.p) + Vehicle (CMC Na, p.o) as SPD group; Haloperidol (0.2 mg/kg, i.p) + Vehicle (CMC Na, p.o), as HPL group; para-Chlorophenylalanine (100 mg/kg, i.p) + OCE (150 mg/kg, p.o); Supiride (50 mg/kg, i.p) + OCE (150 mg/kg, p.o); Haloperidol (0.2 mg/kg, i.p) + OCE (150 mg/kg, p.o).

The interval time between first and second treatment was 30 minutes. mice were then hanged in the TST apparatus for 6 minutes after 30 minutes of the second treatment. Immobility time (s) was recorded at the last 5 minutes of observation.

Data Analysis

All data were presented as mean ± standard deviation (SD). The data obtained was statistically tested using

One-Way ANOVA followed by Tukey's test for multiple comparison. Statistics analysis was performed using Graph Pad Prism software version 9.0. The statistics significance was defined if the p-value < 0.05.

RESULTS AND DISCUSSION

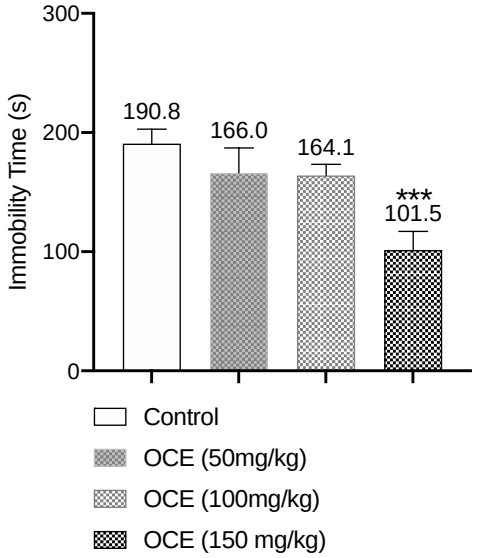

Figure 2. Effect of OCE in TST in mice. *** p < 0.001 compared with control group.

Before the evaluation of possible mechanisms of action in the monoaminergic system, the effective dose determination of OCE as an antidepressant in the animal model TST was performed. As shown in Figure 2., one-way ANOVA analysis showed that OCE was able to significantly lower immobility time (***p<0.001) compared to the control group at a dose of 150 mg/kg, while at doses of 50 and 100 mg/kg there was no significant difference between the OCE group and the control group. These results revealed that *Ocimum sanctum* L. leaf extract obtained by maceration with ethanol 70% can produce antidepressant-like effect on TST model in mice.

Results in Figure 3. indicate the possible mechanism of action in antidepressants-like effect of OCE in serotonin synthesis. In this study, pCPA, a serotonin synthesis inhibitor, was used to investigate the involvement of the serotonergic system on the mechanism of action of OCE as an antidepressant. According to one-way ANOVA analysis, pCPA significantly (*p<0.05) increased immobility time compared to the control group on the TST model in mice. This indicates that pCPA successfully induces the effects of depression through inhibition of serotonin synthesis. Then, pCPA + OCE group also showed significant differences (#p<0.05) to the pCPA group where OCE can decrease the effects of depression induced by pCPA. These results suggest that the

antidepressant-like effect of OCE contribute to the synthesis of serotonin. The mechanism of action of conventional antidepressant drugs is generally based on increased bioavailability of serotonin in the brain (Brunello et al., 2002).

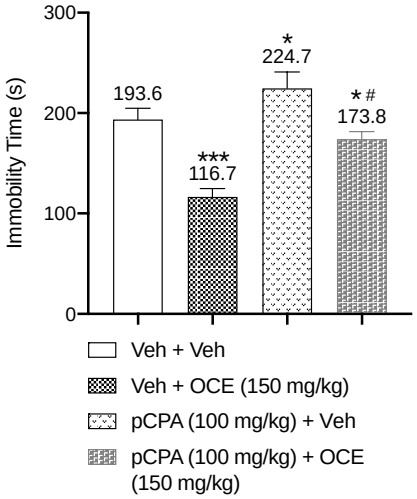

Figure 3. Antidepressant-like effect of OCE involved in serotonin synthesis in TST mice model. *p<0.05, *** p < 0.001 compared with control group, # p <0.05 compared with pCPA group.

The results from Figure 4. and 5. were showed the involvement of dopaminergic system in the antidepressant-like effect of OCE in TST model in mice. In this study, haloperidol (non-selective dopamine antagonist) and sulpiride (selective dopamine D_2 antagonist) were used as the pre-treatment to investigate the contribution of dopaminergic system. As shown in Figure 4., the inhibition of dopamine activity by haloperidol increased immobility time significantly (**p<0.01) compared with control group. Then, OCE can also significantly (#p<0.05) reduced depression-like effect produced by haloperidol. This result indicates that OCE could be interact with dopamine receptor especially on D_1 receptor.

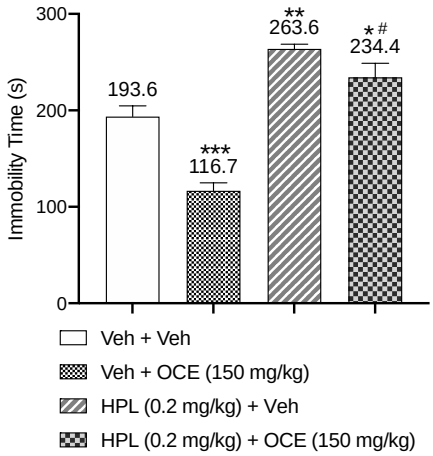

Figure 4. Antidepressant-like effect of OCE involved in D_1 receptor interaction in TST mice model. *p<0.05, **p<0.01, *** p < 0.001 compared with control group. #p<0.05 compared with HPL group.

Figure 5. shows that the antidepressant-like effect of OCE was mediated by D_2 receptor modulation. As we know that OCE alone treated group produced antidepressant-like effect by significantly (***p<0.001)

reduced the immobility time compare to control group. However, the immobility time between SPD group and SPD + OCE group also differs significantly (#p<0.05) where the immobility time of SPD + OCE group was higher than SPD treated group. Therefore, the pre-treatment with sulpiride inhibits antidepressant-like effect produced by OCE. This result indicates that OCE could be interact with D_2 receptor to produce its antidepressants effect.

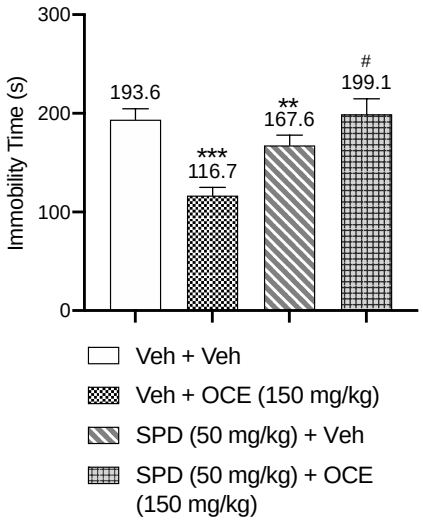

Figure 5. Antidepressant-like effect of OCE involved in D_2 receptor modulation in TST mice model. *** p < 0.001 compared with control group, #p<0.05 compared with SPD group.

Several studies have shown that the effects of antidepressants through modulation on the dopaminergic system are effective for the treatment of depression (D'Aquila et al., 2000; Willner et al., 2005). In addition, antagonist agent D1 and D2 receptors can inhibit the antidepressant effects of some agents on FST and TST models (Machado et al., 2007). Clinical studies also show that D2 receptor agonists are effective for the treatment of depressed patients (Waehrens and Gerlach, 1981).

CONCLUSION

In conclusion, the present study demonstrated that the ethanolic leaf extract of *Ocimum sanctum* L. exhibits an antidepressant-like effect by involving its interaction with dopamine receptors and also plays a role in serotonin synthesis. Altogether, this finding suggest that *Ocimum sanctum* L. may have potential as pharmacological treatment of depression and its antidepressant-like effect mediated by serotonergic and dopaminergic system.

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