

Essential Oil of Patchouli Inhalation Exhibits An Anxiolytic-Like Effect in Mice: Elevated Plus-Maze Test

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Abstract

Anxiety disorder is one of the important public health problems. This study aimed to find out whether patchouli (*Pogostemon cablin* (Blanco) Benth.) essential oil (PEO) has an anxiolytic-like effect in mice. A total of 20 mice were divided into five treatment groups i.e. (C1) was treated with 1 mg/kg of saline intraperitoneally; (C2) was treated with 1 mg/kg of diazepam intraperitoneally; (T1) 1% PEO inhalation, (T2) 2.5% PEO inhalation, and (T3) 5% PEO inhalation. All experimental animals were put in the Elevated Plus-Maze (EPM), observed for five minutes, and investigated the following parameters i.e. the total number of squares that are transverse in the open arms (STO), the number of visits to the open arms (VOA), the number of visits to the closed arms (VCA), the time spent in the open arms (TOA), and the time spent in the closed arms (TCA). In results, T1 and T2 groups had a minor impact on the VOA. T1 group also had little effect on TOA. Closed-arm activities (VCA and TCA) were unaffected by any of the treatments. The conclusion was revealed that T1 or less of PEO inhalation can potentially provide anxiolytic-like effects in mice.

Keywords: anxiety, elevated plus-maze test, essential oil, patchouli, public health

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INTRODUCTION

One-third of the population gets anxiety disorder during their lifetime (Bandelow and Michaelis, 2015). It is becoming a common disorder and an important public health problem in both developed and developing countries (Almeida *et al.*, 2004). DeGrazia and Rowan (1991) stated that, based on neurochemically and behaviorally based evidence, animals could also experience anxious states. Anxiety could worsen physical and mental illnesses and hinder their recovery, and also, highly comorbid with other mental disorders (Bandelow and Michaelis, 2015). The use of anxiolytic drugs currently on the market today involves adverse and undesirable effects (Souto *et al.*, 2011). For example, benzodiazepines have been a mainstay of anxiolytic pharmacotherapy because of their

fast and potent action (Stevens and Pollack, 2005).

Odor can influence psychological and physiological states (Kadohisa, 2013). The study found that essential oils could influence the central neurotransmitter system (Zhang *et al.*, 2016). Approximately 90% of today's global production, 1200–1300 metric tons per annum, of patchouli (*Pogostemon cablin* (Blanco) Benth.) essential oil is realized in Indonesia (Van Beek and Joulain, 2016). Ito *et al.* (2016) found that an extract of patchouli leaves has a sedative effect on mice. However, there are no studies about the behavioral anxiolytic effect of patchouli essential oil.

Pellow *et al.* (1985) found that the elevated plus maze (EPM) test is a simple and one of the most popular methods for assessing the anxiety response of rodents. The test has strong predictive validity, face validity, and construct validity. In

essential oil study, it has been used for testing the anxiolytic effect of such oils as lavender, rose oil, citrus oil, cinnamon oil, and cananga oil (Almeida *et al.*, 2004; Crawley, 2007; Walf and Frye, 2007; Chiocha *et al.*, 2013; Zhang *et al.*, 2016; Sohrabi *et al.*, 2017), but for patchouli essential oil, it has not yet.

The collection of statements above inspires this study to do an experiment that is designed to ascertain the function of patchouli essential oil inhalation as an anxiolytic with an EPM test.

MATERIALS AND METHODS

Ethical Approval

This study was approved by the Animal Care and Use Committee (ACUC) Ethical Clearance No: KE.152.11.2018.

Study Period and Location

The experiment was conducted in February 2019. The EPM test was performed for 4 days around 1–5 pm to minimize the possible influence of circadian alterations (Leite, 2008). This study was conducted at the Animal Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga.

Preparation of Patchouli Essential Oils

Patchouli Essential Oils (PEO) should be diluted before use. PEO was diluted into 3 dosages i.e. (T1) 1.0%, (T2) 2.5%, and (T3) 5.0% as described by de Almeida *et al.* (2004), Leite (2008), and Chioca (2013) by diluting it with propylene glycol. The essential oil was dropped onto the cotton wool just before it was embedded in the inhalation apparatus, and the mice were put inside of it.

Experimental Design

A total of 20 male Swiss albino mice, aged 3–6 months, weighed 25–30 grams were used in this study. Mice from the acclimation cage were randomly divided into five groups i.e. (C1) was injected intraperitoneally with 1 mg/kg of saline; (C2) was injected intraperitoneally with 1 mg/kg of diazepam; the 4 holes in the apparatus wall would be embedded with cotton wool containing 2 ml of (T1) 1% PEO, (T2) 2.5% PEO, (T3) 5%

PEO then mice were put in the inhalation apparatus for 7 min.

The behavioral tests for the C1 and C2 were carried out 30 minutes after the intraperitoneal saline administration. Behavioral tests for the T1, T2, and T3 were performed immediately after the mice were put in the inhalation apparatus.

Mice were placed in the intersection of the four arms of the EPM test, and the behavior was recorded for 5 min because mice demonstrated the most obvious avoidance response in the first 5 min after placement in the elevated open alleys (Walf and Frye, 2007). The activity was recorded using a phone camera. During the test, noise and observer movement should be avoided because mice will terminate their activity and yield a biased result (Sukmanadi *et al.*, 2021).

Behavioral Observation

The ratio of time spent on the open arms to the time spent on the closed arms was observed to assess anxiety (Walf and Frye, 2007). The more time spent and time visiting the open arms indicated the anxiolytic activity (Almeida *et al.*, 2004). The total number of entries was an index of anxiety, and the number of entries and time spent in each arm were indices of primary anxiety. Entry into an arm was counted only when all four paws of the mice were inside the arm (Biala and Kruk, 2008). The total number of squares that were transverse in the open arms (STO), the number of visits to the open arms (VOA), the number of visits to the closed arms (VCA), the time spent in the open arms (TOA), and the time spent in the closed arms (TCA) were noted for subsequent data analysis.

Data Analysis

The difference in means of all treatments was evaluated using Kruskal-Wallis and post hoc Mann-Whitney tests with $p < 0.05$ using SPSS 20.0 for Windows.

RESULTS AND DISCUSSION

The STO result showed that the T1 inhalation group lay between C1 and C2. The same results also happened in VOA and TOA.

Table 1. The mean of STO, VOA, VCA, TOA, and TCA were observed from all treatment groups

Groups	STO	VOA	VCA	TOA	TCA
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
C1	0.75 ^a ± 0.50	0.75 ^a ± 0.50	11.00 ^a ± 0.82	4.00 ^a ± 2.83	150.75 ^a ± 17.44
C2	5.25 ^b ± 1.50	2.25 ^b ± 0.50	9.50 ^a ± 3.11	22.75 ^b ± 10.53	178.25 ^a ± 66.98
T1	2.25 ^{ab} ± 2.21	1.75 ^{ab} ± 1.50	10.00 ^a ± 1.83	14.5 ^{ab} ± 13.53	117.00 ^a ± 48.64
T2	1.75 ^a ± 0.96	1.25 ^a ± 0.50	11.50 ^a ± 1.29	7.00 ^a ± 5.59	174.25 ^a ± 37.69
T3	0.50 ^a ± 0.58	0.50 ^b ± 0.58	9.50 ^a ± 4.79	3.50 ^a ± 4.04	195.50 ^a ± 57.70

^{a,b}Different superscripts in the same column showed significant differences ($p < 0.05$).

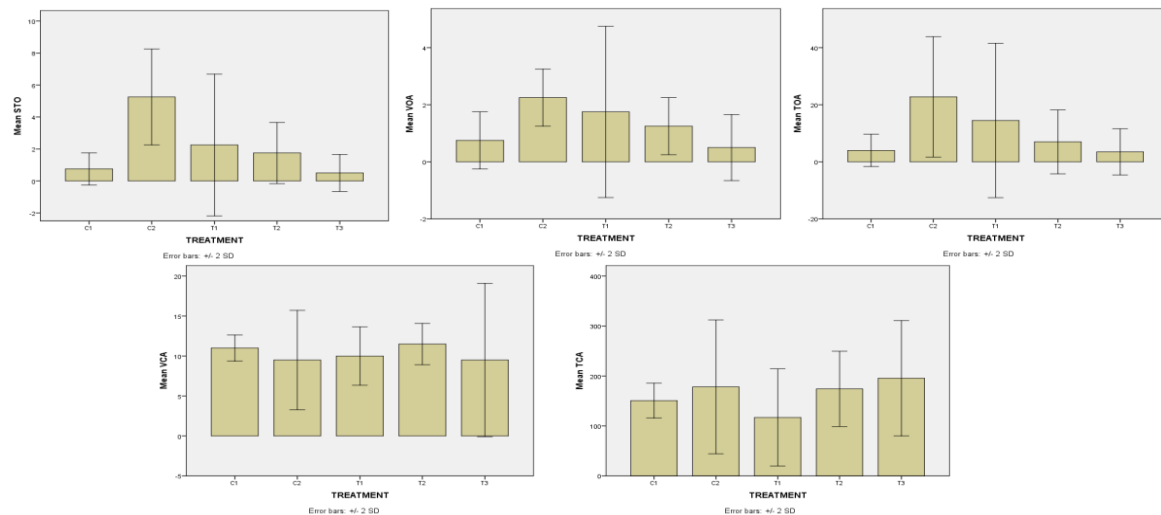


Figure 1. The Mean of STO, VOA, VCA, TOA, and TCA were observed from all treatment groups.

This means that T1 dosage had a small effect on STO, VOA, and TOA. The results of VCA and TCA showed no difference between all the treatment groups, meaning that PEO in all dosages has no effect on VCA and TCA. The results of the data analysis are shown in Table 1 and Figure 1. STO, VOA, and TOA show that as PEO concentrations increase, the effect will be reduced.

Anxiety could be described as psychological, physiological, and behavioral state as a survival urge, even if it was just a potential threat (Steimer, 2002). It is similar but different from fear, which encompasses immediate emotional, physiological, and behavioral responses to a real threat (Hoffman, 2016). As Silva *et al.* (2016) cited, fear is related to anxiety. The apparatus of EPM exploits the conflict between the natural tendency of mice to explore novel areas and their fear of open and high spaces (Walf and Frye, 2007). This fear feeling could induce systematic defensive behavior even if the harm is just a presumption and hasn't been experienced yet. This type of fear is called "innate fear" (Blanchard

and Blanchard, 1989). Anxiety will be expressed by avoiding these places. The EPM test helps assess anxiety by observing the five parameters. Ito *et al.* (2016) found that patchouli leaves contain diacetone alcohol, which is the main active compound with sedative effects. This study also found that there was a point where higher-dosage injection and inhalation of diacetone alcohol showed excitement instead of sedation.

Essential oils can affect a person's psychology and regulate emotions (Qonitallah *et al.*, 2020; Sukmanadi *et al.*, 2021). The molecules of essential oils when inhaled, are directed to the sensory cells of the olfactory scheme. Since each sensor cell flows minutes setae, which record information on perfumes, and transfer it into the center of the brain through the olfactory valve (Bajuber *et al.*, 2020). These cause the release of neurochemical substances, which may be soothing, relaxing, and excitatory or cause euphoria (Puspitasari *et al.*, 2021). Furthermore, aromatic particles enter the nervous system resulting in relaxation, tranquility, and relieve of nerves and hence of nerve centers in the brain.

Due to this anxiety, affective disorders, headaches, and migraines can be addressed in some way (Tripathy *et al.*, 2023).

According to literature and study articles, several essential oils can help in reducing anxiety disorders and as a result, the embodied events that they may cause (Al-Zuhroh *et al.*, 2021). Reported data claim that Piper methysticum and Bacopa Monniera are associated with anxiolytic activity in humans. In another trial on generalized anxiety disorder in a hospital-based clinical setup, Ocimum sanctum significantly attenuated generalized anxiety disorders and also attenuated its correlated stress and depression. As we move on to the literature review and more studies indicates the positive effects that aromatherapy can have in the reduction of anxiety (Fradelos *et al.*, 2015).

Essential oils applied during a massage session were shown to have a positive effect on both levels of anxiety and relaxation over a short-term period. Each client was seen for six aromatherapy massage sessions of 1-hour duration and an initial consultation session. The massage was performed at the same time of day and day of the week for each client in a specific room allocated for aromatherapy use. Each client received the same standardized massage technique from the same therapist although the essential oils were selected and blended specifically for each session/ individual client (Siahaan *et al.*, 2014).

CONCLUSION

It can be concluded that inhalation of PEO at 1% dosage gives an anti-anxiolytic-like effect that was documented by the EPM test. Further study on other behavioral tests is necessary, such as the open-field exploration test, the light-dark exploration test, and the social interaction test, which could complement the results of the study.

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AUTHORS' CONTRIBUTIONS

EPH: Conceptualization and drafted the manuscript. FAKJ, R, NH, KPS, and SAS: Treated the animal laboratory. EPH and SAS: Validation, supervision, and formal analysis. EPH, FAKJ, R and SAS: Performed sample evaluation. NH: Performed the statistical analysis and the preparation of table and figure. All authors have read, reviewed, and approved the final manuscript.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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