EXPLORING ANTIDEPRESSANT POTENTIAL OF PEPPERMINT OIL INHALATION IN STRESS-INDUCED RATS USING HEART ACTIVITY MONITORING

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ABSTRACT

Various studies have been conducted to discover non-pharmacological alternative therapies for mental health disorders such as depression caused by prolonged stress. One function of Peppermint essential oil (PEO) as aromatherapy is to improve mental function and reduce stress. This research objective is to clarify the effectiveness of PEO to balance of brain-body connection. We used rats as animal experiment and using PEO inhalation to explore its potential as an alternative antidepressant therapy, measured by electrocardiogram monitoring using a Wireless Mice Electrocardiogram (WIM ECG) to analyze its impact on the cardiac electrophysiological parameters. Twenty-five male Wistar rats (180-200 g) were divided into five treatment groups: (1) aquadest; (2) 16 µl/h; (3) 40 µl; (4) 80 µl/h; and (5) fluoxetine 14 mg/kg (positive control). All treatment groups were induced with acute restraint stress for 90 minutes along with inhalation process and electrocardiogram monitoring was conducted during the treatment. The result showed, administration of PEO significantly influenced certain cardiac electrophysiological parameters similar to fluoxetine compared to vehicle treatment. Inhalation of 80 µl/h PEO had consistent results that significantly affected the RR interval and heart rate since minutes 30, similar to the result observed with fluoxetine compared to the vehicle ($p<0.05$). Based on these findings, 80 µl/h PEO indicated an antidepressant effect based on the increased duration of the RR interval and the reduction in the number of heart rate (bpm) after PEO inhalation.

Key words: Peppermint Essensial Oil, inhalation, acute restraint stress, antidepressant, electrocardiogram

INTRODUCTION

Stress accumulation may lead to mental health disorders such as depression (Vahia, 2013; Sideropoulos *et al.*, 2022). Nowadays the number of depression cases continues to rise and WHO stated depression as one of the priorities and one of the broadest spectrum of mental disorders (Kemenkes RI, 2017). The number of people suffering from depression has increased

sharply during Covid-19 (Kupcova *et al.*, 2023).

The existence of various stress factors may effect various physiological responses including hormone responses and initiates physiological responses throught autonomic nervous system (ANS), especially to the vital organ of the heart (Golbidi *et al*., 2015; Hinds & Sanchez, 2022). This is characterized by an increase in blood pressure, heart rate, and other cardiac rhythmic parameters that have the potential to cause other cardiovascular disorders (Golbidi *et al*., 2015; Satyjeet *et al.*, 2020).

To alleviate the effects of these conditions, the use of synthetic antidepressant drugs is also increased but that often arrive with broad side effects (Kumar *et al.*, 2012; Yekehtaz *et al*., 2013; Chang *et al.*, 2021; Neumann *et al.*, 2023). However, various studies have been conducted to discover non-pharmacological alternative therapies which one of them is peppermint (*Mentha piperita L*.). Peppermint Essential Oil (PEO) is popularly used as aromatherapy and various alternative medical therapies since it contents of some compounds (Baliga & Rao, 2010; Fung *et al.*, 2021). In addition, the utilization of plant essential oils in aromatherapy has become prevalent for therapeutic purposes and promoting mental tranquility by inhaling them (Lizarraga-Valderrama, 2021).

In this study, Peppermint Essential Oil (PEO) with inhalation method will be used to see its potential as an antidepressant. This study has been focusing on the route of pharmacological transmission, as this is thought to be both persistent and potent. The inhalation method can be delivered through two pathways, (1) olfactory sensory neurons (OSN) and (2) the respiration system, but some volatile components can be delivered directly to the central nervous system (brain) of the target area, which will affect neurotransmitter pathways such as the noradrenergic, 5-HTergic, Dopaminergic, γ-aminobutyric acid-ergic, , and cholinergic (depend on the mechanism of action of each compound) (Kennedy *et al.*, 2018; Masubuchi *et al*., 2019; Fung *et al.*, 2021).

Monitoring of physiological heart function is important in the management of stress and depressive symptoms, especially since there is a tendency to be at risk for heart disease (Golbidi *et al*., 2015; Satyjeet *et al.*, 2020). Using electrocardiogram analysis, the heart activity can be evaluated for changes over time and how certain conditions/treatments may have an effect on it (Kumar *et al*., 2017; Padsalgikar, 2017). Maulana *et al*. (2023) used Wireless Mice Electrocardiograms (WIM ECG) and prove an ECG waveform recording device is non-invasive, affordable, easy to use, and is also utilized for convenient monitoring in rodents such as rats and mice (Nugroho *et al*., 2017; Maulana *et al.*, 2023).

This study aimed to assess the antidepressant effects of inhaling Peppermint Essential Oil (PEO) by analyzing the heart's electrophysiology, an essential physiological marker derived from electrocardiogram (ECG) recordings obtained through wireless mice electrocardiogram (WIM ECG).

MATERIALS AND METHODS

Experimental Animals

Twenty-five male Wistar rats aged 8 weeks (*Rattus norvegicus*) weighing between 180-200 g were housed in the animal facilities at the School of Biological Science and Technology, Bandung Institute of Technology, Indonesia. The rats were raised following laboratory management protocols in a controlled environment with a temperature set at 25±4 °C, relative humidity between 70-90%, and a 12-hour light-dark cycle. Food and water were provided ad libitum to the rats on a daily basis (Liu & Fan, 2017). The animals were allowed to acclimate for a period of 7 days before being divided into five groups: (1) Aquadest (vehicle), 16 µl/h; (3) 40 µl/h; (4) 80 µl/h, and (5) fluoxetine 14 mg/kg (positive control).

Preparation of Peppermint Essential Oil (PEO) and Drugs

The experimental material utilized in this investigation was Peppermint Essential Oil (PEO) sourced from Young Living Singapore Company, comprising 100% essential oil. The essential oil was blended with a carrier substance and subsequently administered through inhalation using a mini diffuser Ultrasonic Ionizer Aromatherapy Diffuser (model KW056-2, ultrasonic frequency 2.5MHz, Nature Co. Ltd), manufactured in China. The groups of PEO were 16 µl/h, 40 µl/h, 80 µl/h, with a vehicle (aquadest) administered via inhalation, and as a positive control Fluoxetine 14 mg/Kg was orally administered 30 minutes before the treatment (Saiyudthong & Marsden, 2011; Chioca *et al.*, 2013; Zhang *et al.*, 2023).

Acute stress induction and PEO inhalation

The induction of stress and inhalation were modified from acute stress procedures describe in previous studies using acute restraint stress for 90 minutes along with the inhalation process (Fukada *et al.*, 2012; Zeldetz *et al.*, 2018; Masubuchi *et al*., 2019). Rats will be placed into an acrylic restrainer measuring 7 cm (diameter) x 20 cm (length) (Figure 1a) and inhaled through a diffuser connected to a funnel that leads directly to the anterior part of the rat (Maulana *et al.*, 2023).

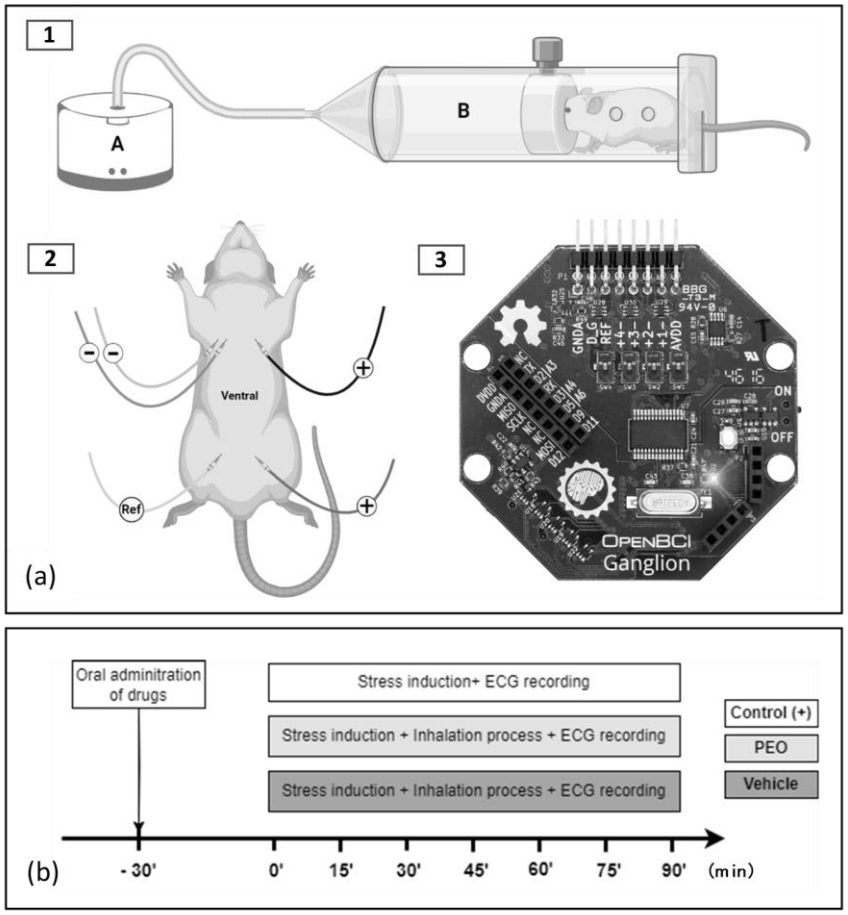


Fig. 1. Stress restraint, inhalation, and ECG recording procedures. (a) 1. The inhalation process uses a diffuser directly connected to the funnel [A], a rat's restrainer the stress induction process through immobilization [B], 2. Positioning electrodes on rats, 3. using OpenBCI software for data recording (Nugroho *et al*., 2017; Maulana *et al*., 2023). (b) Schematic of the time-course of the experiment. As positif control Fluoxetine

was given through oral administration 30 minute prior to stress induction. ECG recording was performed at minutes 0, 15, 30, 45, 60, 75, and 90 (Park *et al*., 2017; Bakhchina *et al*., 2019)

Measurement of ECG Analysis

The assessment of ECG was carried out utilizing the Wireless Mice Electrocardiogram (WIM ECG) apparatus. The ECG features were assessed focusing on intervals (PR, QT, and RR) and heart rate. The ECG recording detection mechanism was adapted from the system pioneered by Nugroho *et al*. (2017) and Maulana *et al*. (2023). The restraint room was modified from previous studies also being used to prevent the movement of the animal model (Nugroho *et al*., 2017; Maulana *et al*., 2023). Electrocardiogram waves were recorded along the treatment process, with a duration of 3 minutes every 15-minute interval up to 90 minutes (Figure 1b). Anesthesia was avoided due to its potential to induce disruptions in heart rate and other electrophysiological parameters (Pereira-Junior *et al*., 2010; Kumar *et al*.,, 2017).

Data Analysis

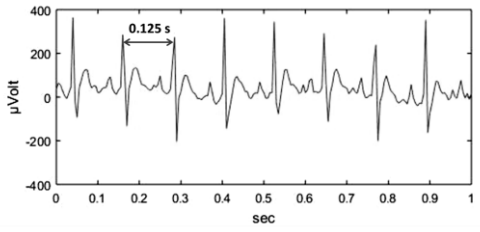
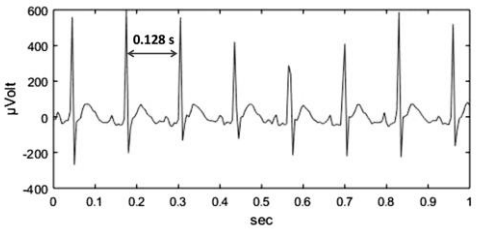
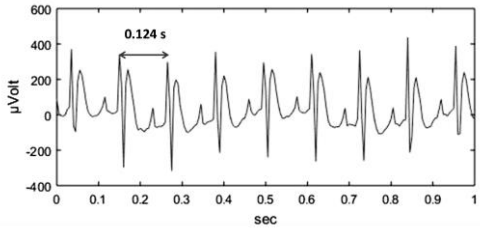
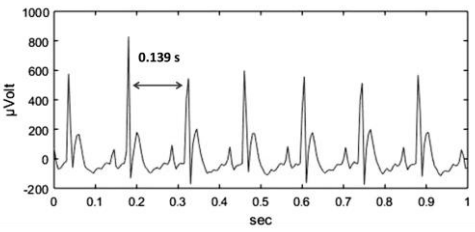
The ECG recording data underwent analysis and visualization through Matlab software. Mean and standard deviation were calculated for the data results. Parametric statistics were conducted using one-way analysis of variance (ANOVA) along with Dunnet's post-hoc multiple comparison, while non-parametric analysis, such as the Kruskal-Wallis test, was also performed. Statistical significance was established when the P-value was below <0.05.

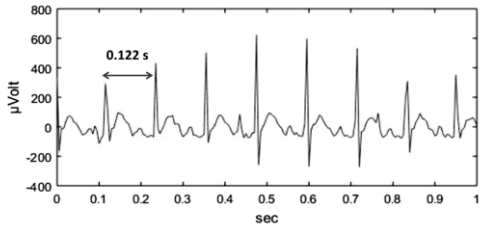
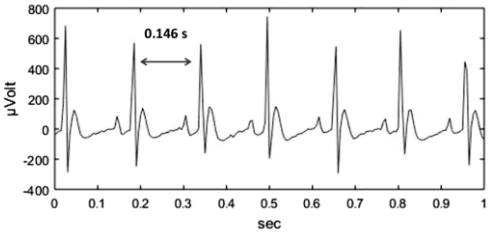
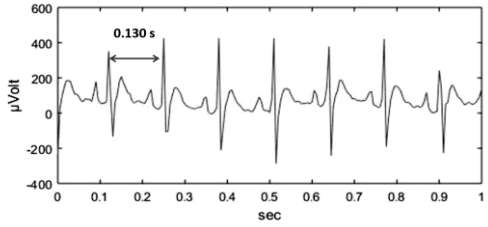
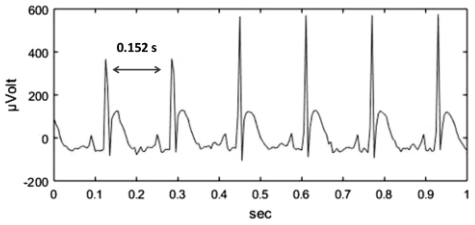
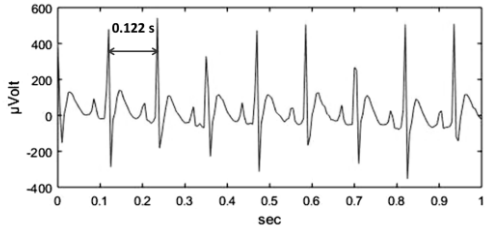
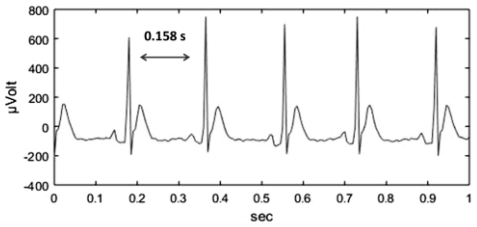
RESULTS AND DISCUSSION

Effects of Stress and Peppermint Essential Oil Inhalation on ECG Wave Characteristics

The ECG waveform characteristics of all treatments were compared within 1 second as shown in Table 1. The vehicle group, showed no difference in the number of peaks and has longer wave density after 90 minutes of treatment. However, when PEO was administered, there was a change in the number of peaks and has shorter wave density, particularly at 80 µl/h PEO. This phenomenon was also seen in the ECG waves after fluoxetine administration.

Table 1. Comparison of electrocardiogram (ECG) waveforms between vehicle (aquadest) group with 16 µl/h, 40 µl/h, 80 µl/h PEO, and fluoxetine administration.

Group	Before treatment	After treatment
Vehicle		
16 µl/h		

40 µl/h		
80 µl/h		
Fluoxetine		

The greater number of peaks and longer wave density indicates the more heart activity cycles and faster of heart muscle works. This will be further identified to determine the duration of each wave parameter especially consists of several ECG waveform parameter such as: RR, QT, and PR interval (Figure 2). The RR interval delineates the heart's activity within a single cycle, measuring from one beat to the next. The PR interval is determined by measuring the time from the start of the P wave to the start of the QRS complex. On the other hand, the QT interval represents the duration of ventricular cardiomyocyte depolarization and repolarization, including the QRS complex and the T wave duration.

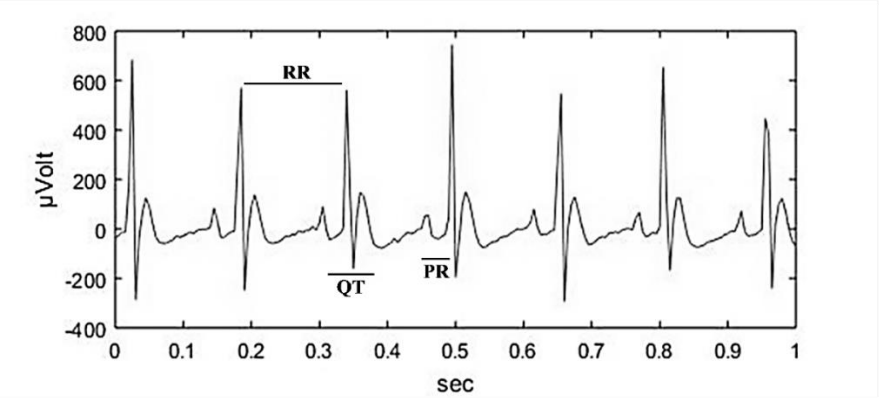


Fig. 2. The identified wistar rat (*Rattus norvegicus*) ECG waveforms; consist s of several ECG waveform parameters: RR, QT, and PR intervals.

Effects of stress and peppermint essential oil inhalation on ECG parameter

Following a 90-minute on ECG recording, the durations of characteristic ECG waves in all groups were assessed based on RR interval, PR interval, and QT interval (Figure 3). The RR interval characteristics in the 80 µl/h PEO group were notably higher when compared to the vehicle group, a trend which was also observed in the Fluoxetine group around 30 minutes after inhalation. Conversely, the PR and QT intervals showed relatively similar patterns across all groups. These results indicate that the inhalation of PEO causes changes in the cardiac system, especially in the function of conducting and contractile muscle cells (Park & Fishman, 2017). Inhaling PEO appears to affect the conducting cells responsible for regulating heart rate, as well as the contractile cells responsible for generating energy for powerful heart contractions that facilitate blood circulation throughout the body (Martini *et al*.,, 2018; Ripa *et al.*, 2023).

The RR interval (Figure 3c) is measured from one of the R peak to the next R peak or indicated as a beat to beat parameter that can be used as a reference for calculating heart rate (Konopelski & Ufnal, 2016; Padsalgikar, 2017). Based on Figure 3c, the average duration of RR interval continues to increase in 80 µl/h PEO treatment as well as fluoxetine group over time. The vehicle treatment showed a stable average duration during the 90-minute treatment of 125-133 ms. The vehicle treatment result will represent a similar condition with stress induction. The previous study reported that the induction of restraint stress showed an average RR interval duration of 125-139 ms (Sgoifo *et al.*, 1997; Bakhchina *et al.*, 2019).

During the PEO treatment, 80 µl/h PEO showed a had consistent results that significantly affected RR interval compared to the vehicle group starting at the 30th, 45th, 75th, and 90th minutes with an average duration of 142, 142, 153, 153 ms (p=0.042; p=0.048; p=0.001; p=0.011). This is similar to the average duration of the fluoxetine treatment which showed an increase in duration from the 30th minute of stress induction but runs about 60 minutes after exposure.

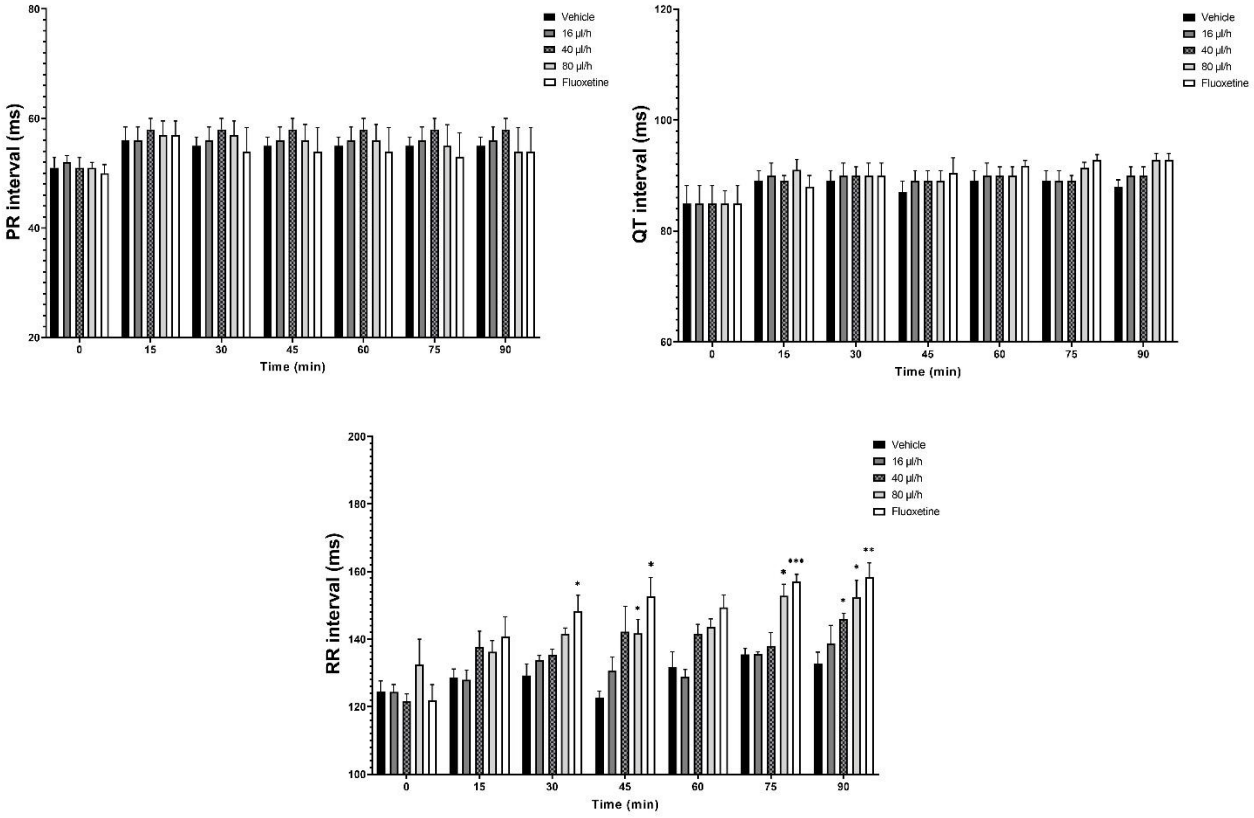


Fig. 3. Comparison of mean (±SEM): a. PR interval, b. QT interval, and c. RR interval duration between Vehicle, 16 µl/h, 40 µl/h, 80 µl/h PEO, and Fluoxetine Groups. The data were analyzed and compared with the vehicle group (One-way ANOVA with Dunnet's post-hoc multiple comparison: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

The increase in duration after PEO inhalation towards the duration of normal RR intervals as previous research states normal conditions obtained from recovery process showed an average duration of 133-150 ms (Bakhchina *et al.*, 2019). Therefore, the induction of stress in rats led to a reduction in the RR interval duration, while following PEO administration through inhalation, there might be an increase in the interval, indicating potentially improved heart function. When compared to the normal duration of the RR interval in rats 118-251 ms, this condition is still within normal limits (Konopelski & Ufnal, 2016).

Effects of peppermint essential oil inhalation on heart rate

Heart rate is obtained in units of beats per minute (bpm) which is calculated based on the RR interval for 60 seconds, using the following equation (Nugroho *et al*., 2017). Heart rate is a condition that can be felt directly by individuals, and represents the working condition of the heart that occurs and can be classified into normal conditions, or abnormal conditions; bradycardia, tachycardia, and irregular (Konopelski & Ufnal, 2016; Padsalgikar, 2017).

$$Heart\ rate\ (bpm) = \frac{60}{RR\ interval\ (s)}$$

A comparison of heart rate in the treatment groups in Figure 4 shown similarity with the RR interval result. There was a constanly decrease in the frequency of heart rate after inhalation of PEO and Fluoxetine. It can also be seen that the average heart rate in the vehicle treatment shows the highest number constantly in the range of 448-495 bpm, this number represents the heart rate of stress inducted rats without PEO inhalation. The previous study states that the range of heart rate in stress induced rats with immobilization stress (ex: restraint stress) shows a range of 450-500 bpm (Yoshino *et al.*, 2005; Bakhchina *et al.*, 2019) and higher than the normal average of 242-452 bpm (Konopelski & Ufnal, 2016).

Induction of acute stress is closely associated with rapid autonomic nervous system (ANS) regulation, as evidenced by sympathetic and parasympathetic activity (Andersson *et al*., 2017). It involves the activity of the neurotransmitter epinephrine and norepinephrine that controls the sympathetic nerves and the parasympathetic nerves that are involved in stress recovery. Norepinephrine increases the speed and strength of heart contractions by stimulating beta-adrenergic receptors in the myocardium (heart muscle), which increases heart rate and cardiac output, thereby increasing the supply of oxygen and nutrients to body tissues (Alhayek & Preuss, 2023).

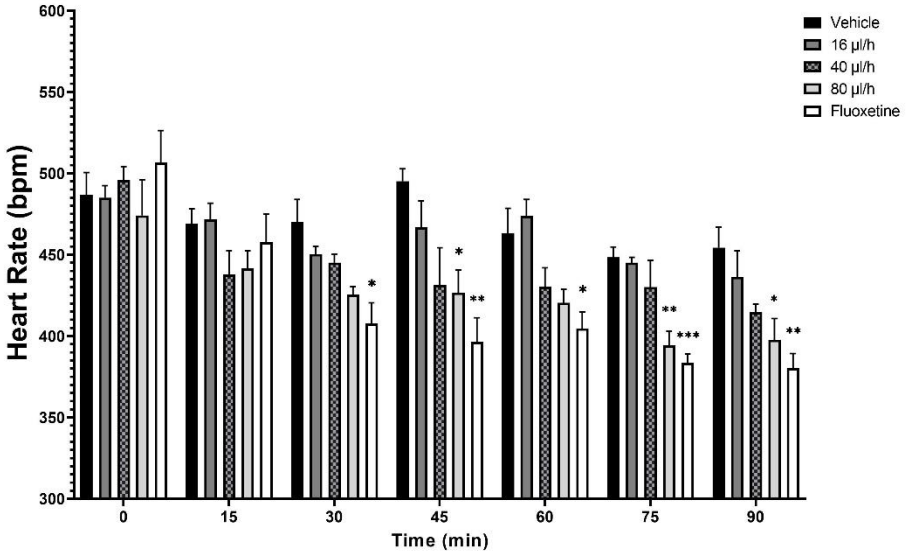


Fig. 4. Comparison of mean (±SEM) heart rate frequency (bpm) between vehicle and 16 µl/h, 40 µl/h, 80 µl/h PEO, and fluoxetine groups. The data were analyzed and compared with the vehicle group (One-way ANOVA with Dunnet's post-hoc multiple comparison: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

While the 80 µl/h PEO group showed a significant decrease in the number of heartbeats at 30, 45, 60, 75, and 90 minutes as related to the RR interval with an average of 426, 427, 420, 394, 398 bpm ($p = 0.009$; $p = 0.022$; $p=0.047$; $p=0.002$; $p = 0.01$); on the other hand, the other groups showed a decrease in the number of beats but did not show a significant difference. This result in line with the use of fluoxetine which has begun to show an effect after 30 minutes.

This condition also showed that PEO inhalation leads to stabilization of heart rate towards normal conditions as previous research showed the similar result with recovery process after stress induction of 350-400 bpm (Bakhchina *et al.*, 2019). The administration of PEO shows a decrease in the number of heartbeats from stress conditions leading to a stable normal heart rate. Other studies have also found the effect of reducing heart rate due to the administration of geranium oil which shows a decrease in heart rate, as well as the administration of dl-limonene which shows a decrease in heart rate to bradycardia (Masubuchi *et al*., 2019; Nascimento *et al.*, 2019).

This result is similar to the administration of fluoxetine as a positive control of antidepressants, and 80 µl/h PEO inhalation has a similar effect to antidepressants in controlling cardiac activity. The inhalation of 80 µl/h PEO began to show an effect after 30 minutes during the treatment, this is different from the fluoxetine treatment which showed an effect after 30 minutes as well but had been administrated orally 30 minutes prior the stress induction began. Thus, the oral administration of fluoxetine showed an effective time to affect cardiac activity 60 minutes after administration.

The inhalation process of essential oils can affect 2 main mechanism pathways; (1) Olfactory Sensory Neurons (OSN) or (2) the respiration system which can directly reach the target area in the brain for small compound (Lizarraga-Valderrama, 2021). In the OSN pathway, volatile compounds in inhaled PEO will through the olfactory impulse delivery pathways. This impulse pathway induces the release of neurotransmitters such as serotonin, and other regulatory systems such as noradrenergic, 5-HTergic, Dopaminergic, γ-aminobutyric acid-ergic, and cholinergic which act as a "bridge" between nerves and other body systems (Kennedy *et al.*, 2018; Fung *et al.*, 2021). In this case, the mechanism of delivery through OSN is relatively faster because it is regulated by the nervous system that works in a matter of seconds-minutes.

Besides through OSN, the inhalation process can also go through the respiration system,

which through the circulatory system during the gas diffusion process that occurs in the alveolus (Fung *et al.*, 2021). This mechanism takes a longer time since the active compounds must be delivered through the blood circulation to the brain. This is also similar to the mechanism of oral administration which will go through a longer process with various series of digestion and then will be absorbed and circulated throughout the body through the blood circulation. However, the use of this type of essential oils will also provide effectiveness because the form of lipophilic nature compound. This substance be more easily transmitted through the blood brain barrier (BBB) and more easily reach the target brain area compared to hydrophilic compounds (Fung *et al.*, 2021; Cui *et al.*, 2022).

CONCLUSION

Based on these results, inhalation of PEO had a real effect on increasing the duration of the RR interval and also decreasing heart rate in rats under stress conditions; This result is similar to the administration of fluoxetine as a positive control for antidepressants, so it can be said that inhalation of 80 µl/h PEO has an antidepressant-like effect in controlling heart activity. This effect demonstrates effectiveness after 30 minutes of treatment and shows a more effective timeframe compared to the administration of fluoxetine. However, there are several limitations in this study, thus requiring further evaluation and testing.

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ETHICAL STATEMENT

This study was approved by ethical committee Ethics Commission of Padjadjaran University, approval number 1148/UN6.KEP/EC/2023.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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