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Original Article

Jasmine essential oil promotes delta-beta power activities in the dorsal hippocampus under slow wave sleep promotion♣

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Abstract

Analyzing sleep electroencephalography (EEG) data can provide insights from application of basic principles of signal analysis (filtering, sampling, and spectral processing). This study investigated whether jasmine essential oil (JEO) intake differed in sleep EEG patterns from sedative drug intake. Adult male Swiss Albino (ICR) mice treated with distilled water, jasmine essential oil and lorazepam administration were assessed for sleep stages offline from the dorsal hippocampal brain activity. Two-way repeated measures ANOVA revealed that JEO reduced the wakening duration while increasing NREM sleep following 60 minutes of intake to the end of the 3-hour recording, in comparison to water gavage. Their pharmaco-EEG fingerprints after a single intake of jasmine oil and lorazepam showed a high power-level of delta and beta frequencies in the first 30 minutes of recording. A dramatic decrease in gamma2 power activity was observed only after lorazepam was administered. Slow wave activity within the hippocampus was a highlight of the scent relaxant as promoter of non-REM sleep.

Keywords: anxiolytic, jasmine, hippocampus, sleep, essential oil, mice

1. Introduction

Sleep cycles in the brains fall into four stages during each period of sleep. Stages 1 through 3 are called non-rapid eye movement (NREM) sleep. Stage 4 or REM sleep is referred to as active sleep or paradoxical sleep. Stage 3 is also known as delta sleep or slow wave sleep (SWS), because it has high levels of slow wave activity (SWA) typically below 4 Hz (Carskadon, & Dement, 2011). Slow wave sleep (SWS) plays an important role in declarative memory consolidation during sleep (Lu, & Göder, 2012; Walker, 2009). During these slow oscillations, excitatory and inhibitory neocortical neurons from all layers in sleeping animals oscillate between depolarized and hyperpolarized states (Chauvette *et al.*, 2010; Timofeev, *et al.*, 2000, 2001). The neocortex generates slow oscillations, and the thalamus maintains them as thalamic inactivation modifies cortical slow oscillations in a temporal manner (David *et al.*, 2013; Lemieux *et al.*, 2014). Sleep slow

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oscillations also play an important role in cortical plasticity (Timofeev, & Chauvette, 2017). It's important to realize that sleep begins with NREM stage 1, transforming into NREM stage 3. NREM stage 2 is then repeated, and finally there is REM sleep. It takes about four to five cycles of repeating each stage during the night (National Institute of Neurological Disorders and Stroke, 2006). Given these differences, it has been commonly assumed that wakefulness, NREM sleep, and REM sleep represent mutually exclusive "global" states.

Jasmine essential oil has widespread applications designated in folk medicine of many countries, due to its multipurpose activities. Evidence has been presented on the effects of the application of jasmine oil in aromatherapy, including improved mood and relief from depression in humans (Hongratanaworakit, 2010; Phuc *et al.*, 2019). Jasmine oil also has a stimulatory effect on the functions of the nervous system. Furthermore, jasmine oil is also used in perfumes and cosmetics, and as an analgesic (mild), antidepressant, anti-inflammatory, antiseptic, antispasmodic, aphrodisiac, carminative, cicatrisant, expectorant, galactagogue, sedative, tonic for (uterine) depression, nervous exhaustion, and stress related conditions (Wang & Weller, 2006). The inhalation of jasmine oil produces an increase in beta wave (13-30 Hz) brain signals in the anterior central and left posterior regions, but no significant changes regarding the power of alpha 1 (8-10.99 Hz) and alpha 2 (11-12.99 Hz) waves in all the regions of the brain (Sayowan *et al.*, 2013). The aroma produces active cortical brain wave responses that are known to vary with extreme sensitivity according to the level of inhalation. However, less research has been conducted on the effects of jasmine essential oil administration on brain function and sleep.

2. Materials and Methods

2.1 Animal studies

Male ICR mice weighing between 25 and 45 g were maintained on a 12 ± 1 h day and night schedule. The animals were fed with a standard diet and water *ad libitum*. The study was conducted according to the guidelines of the European Science Foundation (Use of Animals in Research, 2001) and the International Committee on Laboratory Animal Science (ICLAS) (2004) to minimize animal suffering and the number of animals used. All the procedures were approved by the Animal Ethical Committee of the Prince of Songkla University, Thailand [project license number**:** 2563-01-022].

2.2 The preparation of 0.03% jasmine essential oil

The jasmine essential oil was prepared by steam distillation (Manzan *et al.*, 2003). In brief, steam was forced over jasmine flowers in a still. The hot steam helped release aromatic molecules from the plant material. The molecules of the volatile oils then escape the plant material and evaporate into the steam at a controlled temperature. Afterwards, the steam containing essential oil is condensed in a cooling system, which forms a liquid from which the essential oil and water are separated. In this study, the 0.03% jasmine oil was prepared freshly by mixing 0.1 ml jasmine oil with 30 ml distilled water by vortexing for 10 min.

2.3 Safety evaluation of EOs

An oral toxicity test was carried out on the mice as per the OECD-423 guidelines (Roy & Ghosh, 2005). A dose (2000 mg/kg body weight) of jasmine essential oil was administered orally. The animals were regularly monitored for mortality, clinical signs and changes in body weight daily for 3 days, and were continually observed for a period of 14 days. Signs of acute toxicity included death, fecal boluses, abnormal fur appearance, tremors, twitches, vocalization, gait, and body posture. At the end of the study period, all the animals were subjected to a gross necropsy examination to confirm alterations to the tissues, including the brain, renal organs, spleen and heart. The tissue samples were fixed for 48 h in 10% formalin saline. The tissues were embedded in paraffin and sectioned to a thickness of 5 μm using a rotary microtome. The sections were stained with hematoxylin-eosin (H&E) for light microscopy. The results of the histopathological studies were analyzed visually. The frequency of tissue damage was indicated using the following symbols**:** −, no change was observed in the samples; +, changes were observed in the samples (> 20 changes per vision field) (Zhai *et al.*, 2013).

2.4 The behavioral study on anxiolytic effect

The open field test is used to analyze locomotion, anxiety and stereotypical behaviors, such as grooming and rearing in rodents (Prut, & Belzung, 2003). Changes in locomotion can be indicative of altered neurological processes and may, therefore, reflect changes to brain function. In addition, this test may be used to assess the general health and wellbeing of an animal. Unhealthy animals tend to move less within the area. Mice that are stressed are less active in the open field and display increased stereotypical behaviors (Kalueff, & Tuohimaa, 2004). Such behaviors include those that are repetitive, invariant and seemingly without purpose. Mice that prefer staying close to the walls and travel more within the periphery can be described as showing pronounced signs of anxiety-like behaviors. Mice with lower levels of anxiety tend to spend more time in the central, open area of the box. The total time, speed and distance are recorded and transferred to an open-source toolbox for an automated phenotyping of the mice.

The elevated plus maze (EPM) consists of two open arms crossed with two closed arms. The arms are connected together in a central square. The apparatus was elevated to a height. The animals were treated with jasmine essential oil for the treatment group and distilled water for the control group before being placed individually in the center of the EPM, facing a closed arm. The time spent in both the open and closed arms was recorded (Pellow, & File, 1986).

2.5 Surgery for intracranial electrode implantation and EEG recording

Electrode implantation was performed according to the procedures previously described (Cheaha, & Kumarnsit, 2015). Soon after, the animals were anesthetized with an intramuscular injection of a cocktail mixture of ketamine and xylazine. Once the animal was deeply anesthetized, its head

was fixed with the stereotaxic apparatus. Approximately 15 min after a lidocaine injection was applied under the dorsal scalp as a local anesthesia, the mouse received a midline incision through its scalp. Small holes (2 mm in diameter) were drilled at the top of the skull. Unipolar electrodes were implanted into the dorsal hippocampus CA1 (-2.5 mm posterior to bregma, -1.5 mm lateral to midline, 1.5 mm ventral below the dura) according to the mouse brain atlas (Franklin, & Paxinos, 2019). Ground and reference electrodes were placed over the midline of the cerebellum. Anchored screws were fixed in additional holes for extra stability to keep all the electrodes permanently in place. Next, dental cement was used to fix electrodes and screws onto the skull. The antibiotic, ampicillin, was given intramuscularly once a day for 3 consecutive days to prevent infection. Then, the animals were allowed to recover fully for at least 2 weeks.

The animals were divided into 3 groups. For control group, animals were given distilled water (10 ml/kg body weight) by oral gavage. Animals in jasmine group were given orally jasmine oil (10 ml/kg body weight). The lorazepam (1 mg/kg) dosing was done orally for animals in lorazepam group. EEG and behavioral monitoring were observed for 30 minutes for the baseline in the open field box before administration. Thereafter, the sleep duration effect was monitored for 180 minutes after the administration of treatment. The LFP signals concurrently from the region of CA1 were collected, amplified and digitized using a PowerLab 16/35 system (AD Instruments, Australia) with a 16-bit A/D at a sampling rate of 2 kHz and a bandwidth of 1-200 Hz. Data were stored in a computer using LabChart 7.3.7 Pro software. A notch filtering of 50 Hz was applied to remove the noise from power line frequency.

2.6 Sleep monitoring and vigilance state analysis

The hippocampal EEG signals were then subjected to waveform recognition scoring manually. Vigilant states are automatically classified off-line under three stages, i.e., wakefulness, rapid eye movement (REM), and NREM sleep, according to the standard criteria. Wakefulness is defined by a low-amplitude and high-frequency EEG. REM sleep is characterized by a low-amplitude, high-frequency EEG; the presence of EEG theta-activity (6–9 Hz) in the recording can be used to confirm this state. NREM sleep is commonly defined by a high-amplitude EEG. The presence of high delta activity (0.5–4 Hz) in the EEG is also employed to characterize this state.

To confirm sleep brain activity, HiFI calculations were performed by filtering the EEG signal to 110 Hz and 300 Hz, respectively, to determine sleep scores. A root mean square from time t −0.25 to t +0.25 s for each data point was applied to the filtered signals. The HiFI was computed using the following equation

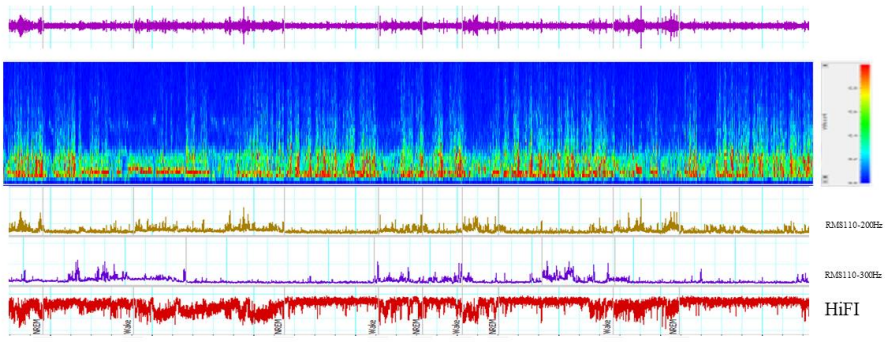
$$\text{HiFi} = \frac{\text{RMS amplitude EEG 110-200 Hz}}{\text{RMS amplitude EEG 110-300 Hz}}$$

Finally, the HiFI signal was smoothed with a time constant of 1 s. A low value of HiFI was observed during wakefulness, an intermediate value during NREM sleep, and a high value during REM sleep. REM was defined as sustained excursions of greater than 4 seconds to higher values. Awaking was determined by excursions exceeding 4 seconds to lower values. Excursions were considered episodes at the beginning. Excursions < 4 s were discarded and merged with the adjacent states. The procedure to score the sleep stage is shown in Figure 1.

For the spectral power analysis, the power spectral density (PSD) was generated by the LabChart software using a Hanning window cosine (window size = 0.976 s, overlaps = 0.488 s). The fast Fourier transform (FFT) algorithm was used for the frequency power analysis. The average of power density was relative to baseline recording and is expressed in discrete frequency bands. In this study, the power spectrum of the hippocampal LFP was specifically divided into delta (0.5-4 Hz), theta (4-6 Hz), alpha 1 (6-8 Hz), alpha 2 (8-12), beta 1 (12-18 Hz), beta 2 (18-30 Hz), gamma 1 (30-45 Hz), and gamma 2 (60-95 Hz) ranges.

2.7 Statistical analysis

The data are expressed as mean ± standard error of the mean (SEM). The statistical significance of treatment choice on sleep-wake profiles and total frequency power activity was assessed by two-way (repeated measures) analysis of variance (ANOVA) followed by the Tukey *post hoc* test. For the anxiolytic study, the t-test was applied. The level of significance was set at $p<0.05$ for all statistical tests.



$$\text{HiFI}=\frac{RMS(110-200\,Hz)}{RMS(110-300\,Hz)}$$

Figure 1. EEG signal representation of HiFi calculation

3. Results and Discussion

To characterize the role of jasmine essential oil in the modulation of the sleep-wake vigilance states, the hippocampal EEG signals over 180 minutes in the jasmine treatment group and in the distilled water control group were analyzed. The distribution and amount of waking, NREM sleep and REM sleep times were evaluated. Under two-way repeated analysis, the influence of essential oils on wake duration ($F_{(2,188)}$=47.835, P<0.001) and NREM sleep ($F_{(2,188)}$=30.522, P<0.001) of mice were addressed. The mice that received lorazepam and jasmine essential oil revealed a reduced time in the waking period (Figure 2A), and an increased time in the non-REM state (Figure 2B) significantly following 30 and 60 minutes after administration, respectively. The significant values are shown in Table 1. It was found that differences were present throughout the entire 180 minutes of recording. No significant difference in the REM was observed among the groups (Figure 2C). Furthermore, duration of sleep including non-REM and REM period following jasmine essential oil intake showed an increase in comparison to control mice (Figure 2D).

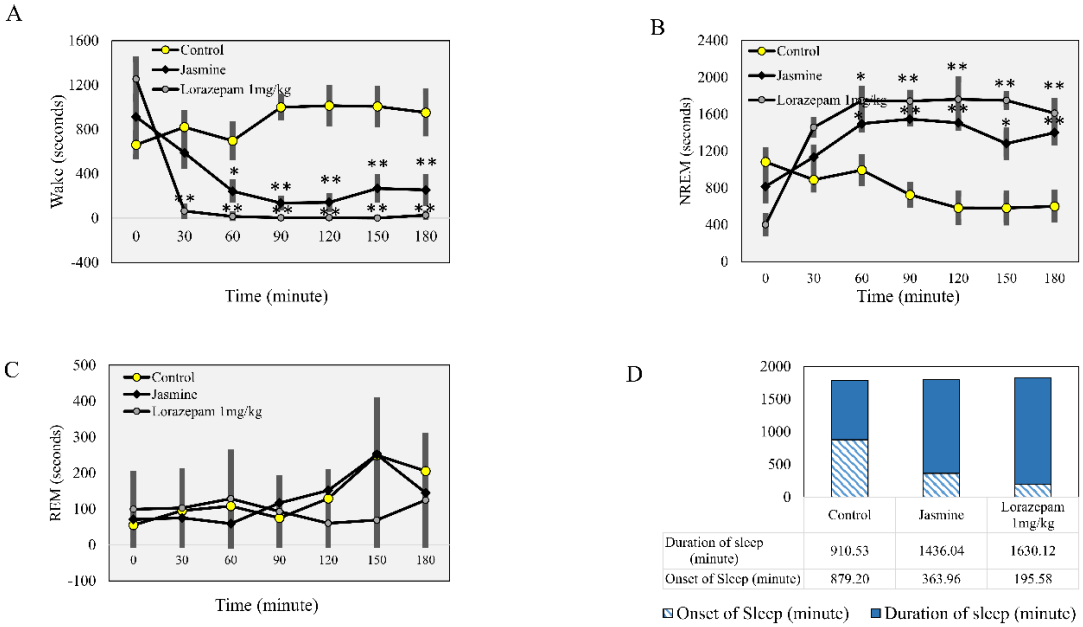


Figure 2. Sleep-wake period characterized to wake (A), non-REM (B), and REM (C). Prominent sleep duration included non-REM and REM sleep as displayed in (D). Data are shown as mean ± SEM. *p<.05 and **p < .001 in comparison to control.

Table 1. The differences of mean value of wakefulness score during baseline and at 180 min following treatment administration

Time	Comparison	Difference of means	p	q	P
Baseline	Lorazepam vs. Control	571.336	3	5.071	0.002
Baseline	Lorazepam vs. Jasmine	323.558	3	2.872	0.110
Baseline	Jasmine vs. Control	247.778	3	2.199	0.270
30min	Control vs. Lorazepam	763.318	3	6.775	<0.001
30min	Control vs. Jasmine	221.194	3	1.963	0.351
30min	Jasmine vs. Lorazepam	542.123	3	4.812	0.003
60min	Control vs. Lorazepam	743.083	3	6.595	<0.001
60min	Control vs. Jasmine	517.722	3	4.595	0.004
60min	Jasmine vs. Lorazepam	225.361	3	2.000	0.337
90min	Control vs. Lorazepam	1003.500	3	8.906	<0.001
90min	Control vs. Jasmine	870.708	3	7.728	<0.001
90min	Jasmine vs. Lorazepam	132.792	3	1.179	0.683
120min	Control vs. Lorazepam	1023.278	3	9.082	<0.001
120min	Control vs. Jasmine	885.306	3	7.857	<0.001
120min	Jasmine vs. Lorazepam	137.972	3	1.225	0.663
150min	Control vs. Lorazepam	947.556	3	8.410	<0.001
150min	Control vs. Jasmine	680.486	3	6.040	<0.001
150min	Jasmine vs. Lorazepam	267.069	3	2.370	0.219
180min	Control vs. Lorazepam	986.528	3	8.756	<0.001
180min	Control vs. Jasmine	760.597	3	6.751	<0.001
180min	Jasmine vs. Lorazepam	225.931	3	2.005	0.335

Analysis of the action of jasmine essential oil on the hippocampal brain region was done by means of the EEG fingerprints of distilled water, jasmine essential oil and lorazepam administrations, shown in Figure 3. Jasmine essential oil developed its action within 30 minutes of ingestion based on changes from baseline of specific frequency. The action of jasmine essential oil was significantly characterized by an increase in the delta ($F_{(5,113)}$=2.930, P=0.016) and beta1 ($F_{(5,113)}$=2.004, P=0.084) powers. During the initial period, delta power increased following jasmine EO intake and the activity was reduced during the entire 3-hour time recording. In mice treated with lorazepam, there is a significant reduction in gamma2 activity over 3 hours. A 30-minute period after drug intake is particularly important for delta and beta power. The regression model estimates of gamma2 in the dorsal hippocampus induced by lorazepam were consistent with decreased brain activity in Figure 4.

Delta power activity in the cortex has been viewed as a measure of intensity of NREM sleep in rodents (Mendelson, & Bergmann, 1999). Delta activity is correlated with a suppression of gamma activity found in NREM sleep (Siclari *et al.*, 2014). There was a greater amplitude of delta waves in NREM than in REM sleep, and the amplitude decreased over subsequent sleep periods. Slow wave activity (SWA), or delta power, is commonly used as an indicator of sleep pressure and is homeostatically regulated (Feinberg, 1974). Slow wave generation and spreading across medial-occipital clusters is affected by changes in synaptic strength and density resulting from neuromodulatory processes and sleep (Riedner *et al.*, 2007; Tononi, & Cirelli, 2014). This possibility is also in part supported by the observation of delta increase in initial time after jasmine essential oil intake, associated with increased rate of inhibitory brain process. Based on prior studies, patients with primary insomnia demonstrated significantly elevated spectral power of the EEG beta during NREM stage 2 sleep suggesting wake promotion in cortical network (Perlis *et al.*, 2001; Spiegelhalder *et al.*, 2012). In terms of a relationship between cortical and autonomic activation during NREM sleep, beta power was found to highly correlate with parasympathetic modulation (Kuo *et al.*, 2016). Changes in the beta frequency band caused by jasmine essential oil intake presented in averaged spectrum in some periods. In several key aspects, these findings parallel the recent observation of distinct slow-wave populations of NREM sleep, which are temporally related to high-frequency increases (beta activity). Consequently, delta and beta waves are more active during non-REM sleep, which stands out against the background of calmness and drowsiness.

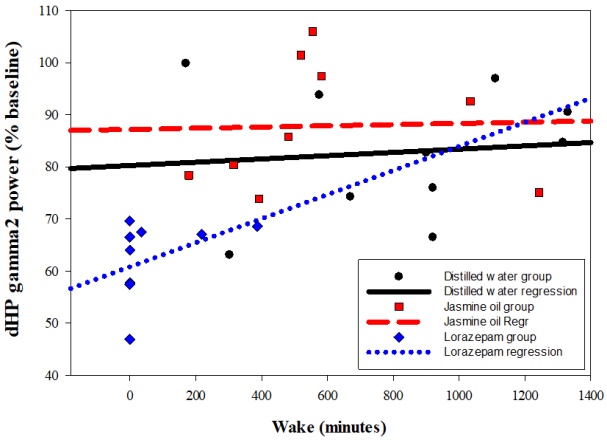


Figure 4. Regression model estimates of gamma2 in the dorsal hippocampus induced by lorazepam were consistent with decreased brain activity.

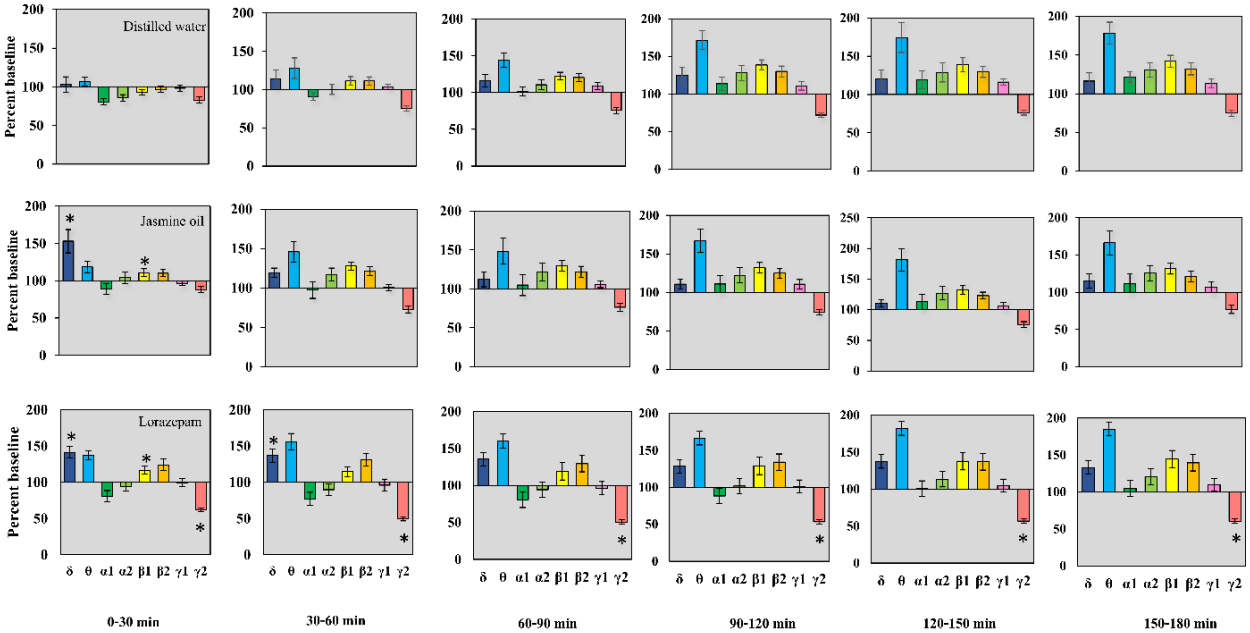


Figure 3. Comparison of changes in the frequency content of dorsal hippocampus of distilled water control (n=10), jasmine essential oil (n=9) and 1mg/kg lorazepam (n=7) administration groups. Data were given in power density in 30 minutes intervals over 180 minutes of recording. Bar graphs display means ± SEM. *p<.05 and **p < .001 in comparison to control.

The benzodiazepine medication lorazepam is commonly used as a sedative and an anxiolytic and can also produce more sleep, as pharmacological alpha suppression was observed in human EEGs. The sedative and anxiolytic drug mechanism of action is by binding to the benzodiazepine receptors in the post-synaptic GABA-A ligand-gated chloride channel at different sites of the central nervous system (CNS), leading to an increased flow of chloride ions into the cell causing hyperpolarization of the cellular plasma membrane (Y.-T. Wang *et al.*, 2022). In Alzheimer's disease, it helps treat insomnia and anxiety disorders (Louzada *et al.*, 2022). As hypnotic drug properties, the adverse effects of benzodiazepines are attributed to strong sedation and often they impact cognition and psychomotor performance (Dumas, 2022; Subhan *et al.*, 1986). The classification of wake-sleep stages in lorazepam treatment group revealed significantly decreased stage 1, increased stage 2, and no change in stages 3–4 of sleep in comparison to placebo (Roth *et al.*, 1980). Under behavioral study, treatment with 2 mg lorazepam (both single and multiple doses) markedly increased light sleep, and resulted in a widespread impairment of choice reaction time, tracking accuracy, threshold reporting of sedation, a reduction of sleep onset latency, and improved sleep quality (Tan *et al.*, 2019). Despite increasing the total sleep time, lorazepam impaired sleep dependent learning and increased next day impulsivity, suggesting the possibility of different, sleep-related cognitive effects of mechanistically distinct GABAergic sedative hypnotics (Morgan & Malison, 2008). Lorazepam had a marked effect on the brain waves, significantly increasing power in the slow (1-7 Hz) and some fast (13-20 Hz and 21-30 Hz) wavebands whilst reducing power in the mid-range (8-12 Hz) (Link *et al.*, 1991). In addition, the reduction of alpha power exclusively correlated with the lower thalamic activity mediated by corticothalamic loops (Schreckenberger *et al.*, 2004; Yeum, & Kang, 2018). There is a consistent relationship between alpha and gamma2 suppression over 3 hr.

The effect of jasmine oil inhalation in humans showed the significant power increase in beta (13-30Hz) across the cortex (Sayowan *et al.*, 2013c). According to the present study, beta band activity increased in the prior 30 min after intake of jasmine oil and was related to the brain inhalation processes. As a result of jasmine essential oil intake, lorazepam showed increased delta and beta activity, but no scent. The antidepressant activity of jasmine essential oil was discussed in the inhibition of the central nervous system. The increase in slow delta and beta frequencies in dorsal hippocampus, the increase of time spent in areas where positive emotions are expressed of an open field and elevated plus maze were all consistent with the slowing down of brain activity following short periods of jasmine essential oil intake. Considering the speculation regarding brain waves associated with sleep-wake cycles, jasmine essential oil consumption is not different than inhalation, especially to the dorsal hippocampus. Further study is needed to investigate how jasmine essential oil affects brain sleep in other brain regions.

A 15-minute open field test (OFT) was conducted for mice treated with jasmine essential oil, with mice were given distilled water as the control, to confirm the anxiolytic effect in association with slow wave sleep promotion. The locomotor tracking showed that the mice that received 0.03% jasmine essential oil explored more in the central arena of the apparatus than the control group (Figure 5A). The significant effect was seen when the activity was calculated according to the total time index in the center and corner zones (Figure 5B). No significant difference was detected in average speed

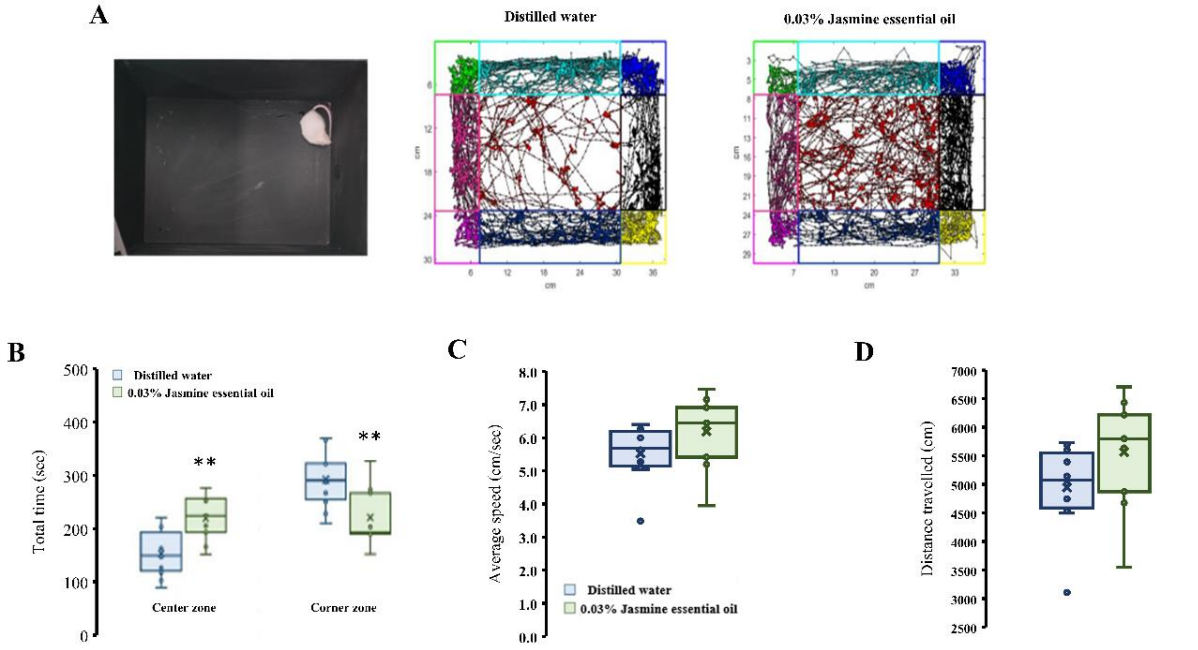


Figure 5. Locomotor activity response to jasmine essential oil in open field apparatus. (A) After treatment by forced feeding, the animal movement was tracked minutes in the chamber. Total time spent traveling to the center and corner zones (B) during the open field monitoring. Average speed (C) and distance traveled (D) during active phase in the apparatus was compared between treatment groups. Data are shown as mean ± SEM. $^{**}p < .001$ in comparison to control. $N = 4–7$ mice/ group.

or distance travelled (Figure 5C-D). The anxiolytic effects on the exploratory activity were also observed in the elevated plus maze (EPM) from both the light/dark transitions for 5 minutes. Also, consistent with the results from the OFT, the mice that received 0.03% of jasmine oil explored more in the open arms (OA) and central area (CA) than the control mice (Figure 6A). The study of the anti-anxiety effect from the consumption of jasmine essential oil showed that a significant amount of time was spent in a specific area. The total time spent in the closed arms was significantly lower in the jasmine oil gavage group than in the distilled water control group (Figure 6B). The animals that had received the essential oil spent more time in the central arena (Figure 6D). There were no significant difference in the time spent in the open arms (Figure 6C), in average speed or in distance travelled between the groups.

The increase in movement of the mice to the central arena in the open field test after the administration of jasmine essential oil indicated a natural anti-anxiety effect. The experimental animals were typically considered to be less anxious than the control animals as they entered the central arena more often than the latter (Seibenhener, & Wooten, 2015). The elevated plus maze (EPM) test is one of the most popular tests available for the screening anxiolytic drugs in animal models (Crawley, 2012; Rodgers *et al.*, 1997). The total number of entries score and the total distance are considered useful indexes of general activity. Total entries score is also an index of anxiety, and time spent in each arm constitutes the index of primary anxiety (Korte & de Boer, 2003). The avoidance of the open arms is considered to be a result from the induction of higher levels of fear. It is thought that the aversion of mice to explore the open arms of the maze is caused by fear of open and elevated spaces. A systematic review of the effects of jasmine extract stated that inhalation of jasmine essential oil could produce anxiolytic activity and also had stimulatory properties (Ilmberger *et al.*, 2001; Rahman *et al.*, 2013). The effects can be determined by odor-induced alterations in a negative slow potential occurring during the period between warning and imperative stimuli or contingent negative variation (Torii *et al.*, 1988). Jasmine oil is beneficial in the treatment of severe depression and soothes the nerves, producing a feeling of confidence, optimism and euphoria, while revitalizing and restoring energy and improving memory (Holmes, 1998). The main chemical components of jasmine oil include Benzyl acetate, β – linalool, Benzyl propionate, and volatile oils of carminative, aromatic, antispasmodic, antidepressant, antimicrobial, astringent and stimulatory types. The jasmine odor is associated with alertness which ranged from sleep to wakefulness in sedated stage using pentobarbital (Tsuchiya *et al.*, 1992). However, relaxant activities have been reported on the guinea-pig ileum and rat uterus *in vitro* (Lis-Balchin *et al.*, 2002) and jasmine lactone odor increased the amounts of alpha and theta waves which suggested a relaxing effect of the odor (Hongratanaworakit, 2004). Therefore, the preference of time in central zone in the elevated plus maze after jasmine essential oil intake could be a sign of reduced stress from being high from the floor, as found in research associated with jasmine odorant. These behavioral studies confirmed that jasmine essential oil intake can improve negative emotions.

The jasmine essential oil might cause toxicity. Therefore, a single high dose of jasmine essential oil (2,000 mg/kg) was given to 8 representative mice daily for 3 consecutive days to assess the toxicity. Their symptoms and body weights were observed for 14 days. The results showed no signs of acute toxicity as regards death, skin, fur, eyes,

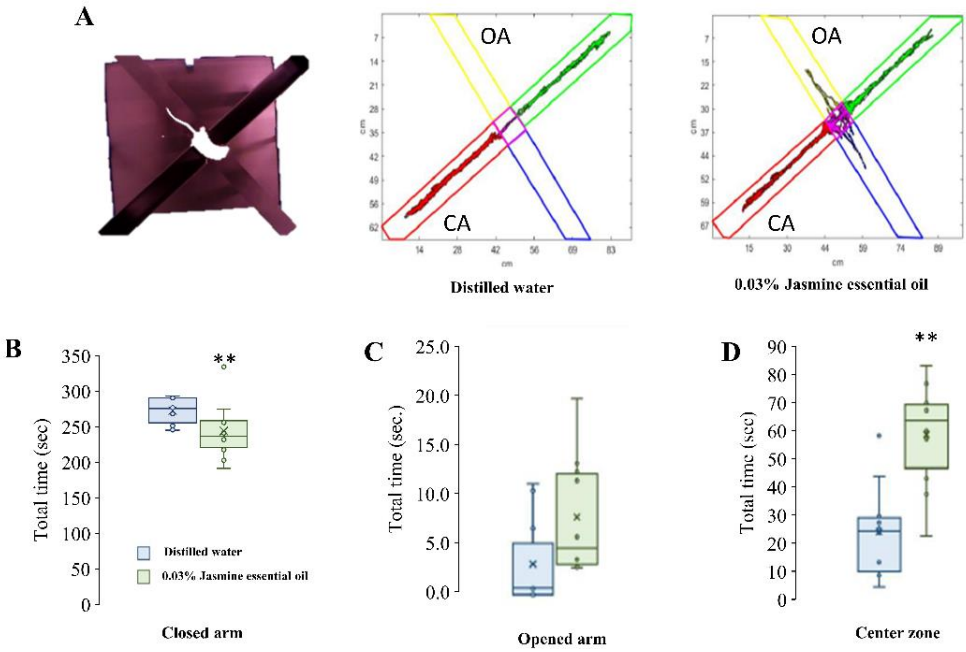


Figure 6. Mice treated with distilled water or with 0.03% jasmine essential oil were evaluated in elevated plus maze (EPM). On test day, mice given jasmine essential oil frequently access the open zone of the maze. Data are expressed as total time spent in preference zone, the closed arm (B), opened arm (C) and the center arena (D). Bar graphs display means ± SEM, with points representing individual values. $^{**}p < .001$ in comparison to control.

movement, respiration, tremor, convulsion, lethargy, sleep, coma, or diarrhea (data not shown). A light microscopy examination of 4 specific brain regions (cerebral cortex, hippocampal CA1, hippocampal dentate gyrus and cerebellum), liver, renal, spleen and heart tissues revealed no histopathologic effects from the acute oral gavage of jasmine essential oil (Figure7 A-B). A high dose of jasmine essential oil did not cause morphological changes or cellular damage in these organs.

4. Conclusions

In summary, jasmine essential oil administration could pass directly right to the brain via the pathway of the nostrils or digestive tract, to have a direct effect on the areas of the brain that control feelings of stress and anxiety. Physiologically, jasmine essential oil triggers slow delta and beta frequency oscillations in the hippocampal area to promote NREM sleep stage. As opposed to lorazepam's alpha and gamma2 suppression, the administration of jasmine oil induces sleep by exerting a relaxing effect instead of sedation. There is a possibility that this effect could be used as an alternative remedy for anxiety and for insomnia.

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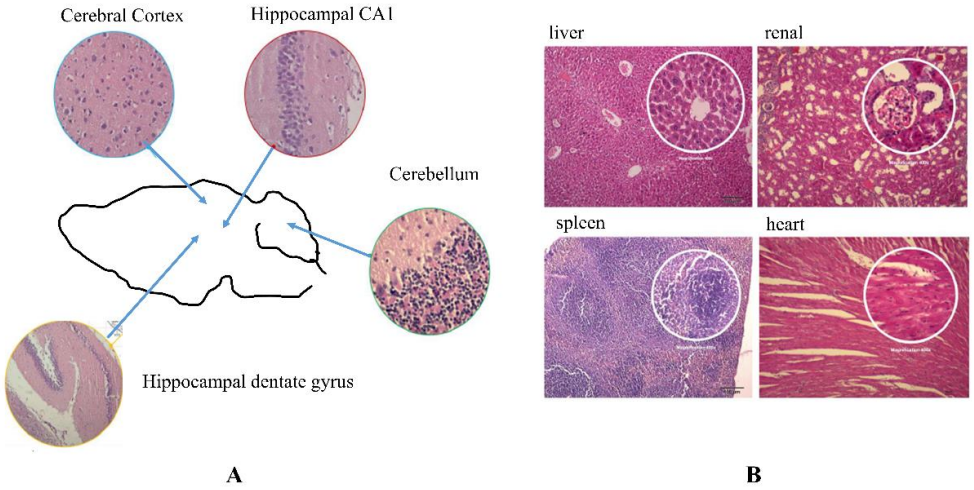

Figure 7. Histological samples. Hematoxylin and eosin stained brain tissues (A) and organs including liver, spleen, renal and heart (B) after acute 2000 mg/kg jasmine essential oil intake, assessed under a light microscope (40x). At the 100x magnification shown in the circles, no toxic effect was found.

Table 2. The differences of mean values on non-REM scoring during baseline and at 180 min following treatment administration

Time	Comparison	Diff of Means	p	q	P
baseline	Control vs. Lorazepam	759.639	3	5.916	<0.001
baseline	Control vs. Jasmine	257.639	3	2.007	0.335
baseline	Jasmine vs. Lorazepam	502.000	3	3.910	0.018
30min	Lorazepam vs. Control	380.417	3	2.963	0.096
30min	Lorazepam vs. Jasmine	133.722	3	1.041	0.742
30min	Jasmine vs. Control	246.694	3	1.921	0.366
60min	Jasmine vs. Control	461.792	3	3.596	0.033
60min	Jasmine vs. Lorazepam	92.153	3	0.718	0.868
60min	Lorazepam vs. Control	369.639	3	2.879	0.109
90min	Jasmine vs. Control	775.194	3	6.037	<0.001
90min	Jasmine vs. Lorazepam	5.111	3	0.0398	1.000
90min	Lorazepam vs. Control	770.083	3	5.997	<0.001
120min	Lorazepam vs. Control	1195.444	3	9.310	<0.001
120min	Lorazepam vs. Jasmine	288.097	3	2.244	0.256
120min	Jasmine vs. Control	907.347	3	7.066	<0.001
150min	Lorazepam vs. Control	997.889	3	7.772	<0.001
150min	Lorazepam vs. Jasmine	334.917	3	2.608	0.160
150min	Jasmine vs. Control	662.972	3	5.163	0.001
180min	Lorazepam vs. Control	810.861	3	6.315	<0.001
180min	Lorazepam vs. Jasmine	11.403	3	0.0888	0.998
180min	Jasmine vs. Control	799.458	3	6.226	<0.001

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