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The effects of sweet almond oil inhalation on brainwave activity

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ABSTRACT

Sweet almond oil is a fixed oil obtained from the kernel of *Prunus dulcis* (Mill.) D. A. Webb. It is widely used as a carrier oil, or a diluent of essential oils, in aromatherapy. In an A-B experimental study of essential oil inhalation that considered the applicability of sweet almond oil as a diluent, sweet almond oil was repeatedly administered to participants. This study aimed to investigate the effects of inhaling sweet almond oil at two different times on brain wave activity, using the A-B experimental design. Twenty-five healthy participants ranging from 20 to 35 years of age were recruited. Sweet almond oil was administered by inhalation to each participant twice, with a 7-day washout period in between treatments. The absolute powers of delta, theta, alpha, and beta brain waves were recorded using a Nicolet EEG v32. Results from the EEG recordings showed no significant differences between brain waves after both the first and second inhalations of sweet almond oil. In conclusion, sweet almond oil was found to be suitable for use as an essential oil diluent.

Key words: brainwaves, electroencephalography, sweet almond oil

INTRODUCTION

Herbal medicine, or phytomedicine, refers to using plant parts for medicinal purposes[1]. Recently, herbal medicine has regained its popularity as part of an herbal renaissance because more people have turned to herbal medicines or products to maintain their health. Herbal medicines have been shown to effectively treat illnesses and promote health. Globally, more than 53,000 species of herbs and plants are used in herbal medicine[2]. Thailand, moreover, has relied on herbal medicines and treatments since ancient times. Initially part of the country's folklore, herbal medicine has since been incorporated with traditional medicine. The

use of plant parts has been widely administered and recommended.

Alternative medicine is defined as the treatment or prevention of diseases through the use of herbal medicines and local wisdom. Traditional Thai medicine is a beneficial option within the primary health care system and is mostly based on the use of herbal medicines, especially aromatic plants. These plants can produce essential oils, which are a mixture of volatile substances extracted from plant parts, such as leaves, stalks, flowers, roots and resins[3]. Essential oils are often used in herbal medicine. In particular, the practice of aromatherapy intentionally relies on essential oils to promote and improve health, well-being, and hygiene[4]. Various essential oils are considered healthful and are used to treat or restore mental and physical balance[5]. Previous research has indicated that essential oils can help to relieve stress, anxiety, depression, and other mood disorders[6]. The inhalation of essential oils transmits signals throughout the olfactory system, stimulating the brain to produce neurotransmitters[7]. Olfactory stimulation is a mechanism of action through inhalation. In human and animal models, the reported physiological effects of inhaled essential oils include sedation, alterations in mood, improved neurotransmission, and emotional heightening *via* the amygdala. Most essential oils are heavily diluted in a carrier oil, which is considered to be inert and is used only for dilution purposes. Common carrier oils include canola, sunflower, olive, jojoba, and sweet almond oils[8].

Sweet almond oil is a fixed oil obtained from the kernel of *Prunus dulcis* (Mill.) D. A. Webb, in the Rosaceae family. It is an unsaturated oil, with oleic acid (O, C18:1) being the main fatty acid[9]. Sweet almond oil is popularly used as a carrier oil or base oil for essential oil dilution[6,10]. It is also used as a control in research that considers different essential oil administration routes, including transdermal absorption and essential oil inhalation. For example, in a previous study that focused on transdermal absorption, sweet almond oil was used as both a diluent and a control and compared with sweet orange oil to measure the effects of sweet orange oil as an aromatherapeutic intervention in humans[11]. Other clinical studies on the effects of essential oil inhalation have commonly conducted placebo-controlled trials. In these studies, the participants were randomly allocated to receive essential oil (diluted with sweet almond oi)) or sweet almond oil (the diluent). The outcomes were then compared to assure that the effects were from the essential oil. For example, Joulaeerad *et al.*, (2018) studied the effect of peppermint oil aromatherapy on the severity of nausea and vomiting during pregnancy. Peppermint essential oil was diluted with sweet almond oil and was administered by inhalation to the intervention group. Sweet almond oil was administered in the same way in the control group as a placebo. The researchers reported that the effect of aromatherapy with both peppermint oil and the placebo were the same in this study, possibly because of the psychological effects of aromatherapeutic intervention on pregnant women[12]. Heydari *et al.* (2018) investigated the effects of aromatherapy with *Citrus aurantium* blossom essential oil on premenstrual syndrome in university students. The participants in the intervention group were asked to inhale *Citrus aurantium* essential oil diluted with sweet almond oil, while the participants in the placebo group were asked to inhale sweet almond oil. The researchers concluded that inhaling *Citrus aurantium* essential oil was effective in relieving the

psychological symptoms of premenstrual syndrome, and it also led to a more noticeable decrease in physical symptoms compared with the placebo group, in which sweet almond oil inhalation did not cause any significant changes[13]. This type of experimental design requires large sample sizes due to the existence of both within- and between- subject variations. Sample sizes can also be large when the desired effect size is small[14].

The A- B experimental design is another type of clinical study, in which each participant sequentially receives both treatments. With this design, the major concern is that the residual effect of the first period treatment continues into the second period treatment. Sayorwan *et al.* (2012) used the A-B design on studies concerning the effects of lavender oil and rosemary oil inhalation on emotional states, autonomic nervous system activity, and brain electrical activity. The findings revealed that lavender oil inhalation produced a relaxing effect, while rosemary oil inhalation produced a stimulating effect. However, sweet almond oil also showed some significant changes in both emotional states and the activities of the nervous system[15-17].

Brain wave activity is evaluated by electroencephalogram (EEG) and used as a physiological indicator of the effects of aromatics. EEG recordings show brain wave responses expressed in brain wave amplitude and frequency. Brain waves can be divided into four basic groups. Waves with a frequency of 8- 13 Hz are alpha waves, those with a higher frequency are beta waves (15–30 H)), and those with lower frequencies are either theta or delta waves (4–7 Hz and 0-4 Hz, respectively). Alpha waves are dominant during mentally relaxed states, while beta waves are dominant during periods of concentrated thought or tense mental states in response to a stimulate[18].

In essential oil studies that follow the A-B design and use sweet almond oil as a control, treatments with the essential oil also used sweet almond oil as the diluent. Then, sweet almond oil was repeatedly administered. For instance, a previous study chose sweet almond oil as a diluent and a control to determine olfactory thresholds because it was a non-olfactory stimulating diluent[19]. A large number of previous studies have been conducted using sweet almond oil as a diluent. However, no known research has investigated the effects of sweet almond oil as an intervention and a control on brain wave activities. Therefore, this study aimed to determine whether twice inhaling sweet almond oil affected brain wave activities, using an A- B experimental design and EEG recordings.

METHODS

Study design and setting

In this study, the A-B experimental design was used. Each subject received sweet almond oil twice. The research was conducted in the mornings to avoid circadian variation at the Kanchanabhisek Institute of Medical and Public Health Technology.

Participants

Twenty- five healthy participants were recruited from the general public, based on the recommendations of Lemeshow (1990), and Sayorwan (2011) [20,21]. The study calculated a significance level lower than 0.05 (Z_{α}= 1.96), a test power of 80%, and a drop-out rate of 10%.

Ethical considerations

This research was approved by the Ethics Review Committee for Research Involving Human Research Subjects, Health Science Group, Chulalongkorn University on February 8, 2018, and given the ethics number COA No. 034/2561. The clinical trial registry number is TCTR20181108002 The researchers received the participants' written informed consents after registering them in the study.

Research instruments

The researchers utilized a Nicolet EEG v32 from the Natus Neurology Company, USA, for brainwave recordings and placed the set of 21 electrodes with 1 additional ground based on the international 10-20 system at Fp1, Fp2, F3, F4, F7, F8, Fz, C3, C4, Cz, P3, P4, Pz, T3, T4, T5, T6, O1, O2, A1 and A2 (LOC and ROC to track eye movements). The power (an amplitude of 2) was calculated for each frequency band (delta, theta, alpha, and beta) during the resting state and two periods of sweet almond oil administration. The areas of interest were grouped into the left anterior area (Fp1, F3, F7), right anterior area (Fp2, F4, F8), left posterior area (P3, T5, O1), right posterior area (P4, T6, O2), and the central brain regions (FCz, Cz, CPz).

The natural sweet almond oil used in this study was obtained from the Thai-China Flavours and Fragrances Industry.

Each participant was asked to wear a face mask and inhale one ml of sweet almond oil. The oil was delivered from an oxygen pump system through a plastic tube in an inhalation set for adults, which allowed selective routine air flow at 2 L/min.

Inclusion and exclusion criteria

Healthy participants between 20 and 35 years old with an average body mass index and normal cardiovascular health based on WHO and Asian criteria values were tested for a normal sense of smell by the n-butyl alcohol test[22,23]. Participants had the following characteristics in common: they were right-handed, as measured by the Edinburgh Handedness Inventory scale[24]; they were non-smokers; they had never lost consciousness longer than 30 minutes; they had no symptoms of upper respiratory infections, hypertension, or cardiovascular disease; and finally, they had no history of neurological illness or epilepsy. Before the experiment, the participants were instructed not to use hairspray, deodorants, or perfume, smoke cigarettes, or drink any caffeinated beverages. Additionally, they were told they should not be tired or drowsy on the day of the experiment.

Research Procedures

Each experiment was conducted in a quiet, air-conditioned (24 ± 1 °C) and pre-ventilated room with 50- 65% humidity between 8. 00 a. m. and 12. 00 p. m. Participants were tested separately to avoid contamination. EEG recordings were taken in the following order: at resting condition (baseline) for 5 min with the participants' eyes opened and then for 5 min with their eyes closed; at the first inhalation of sweet almond oil for 8 min with their eyes closed (SO1); and then, after a 5 min interval[19], at the second inhalation of sweet almond oil for 8 min with their eyes closed (SO2).

Data analysis

The data was computed and analyzed using STATA statistical software, version 14. A paired t-test was used for the comparison between periods within participants.

RESULTS

Demographic data of the participants

Twenty- five females with an average age of 20.2 ± 0.4 years and a normal body mass index (20.3 ± 1.2) participated in this study (Table 1). There were no significant differences between the band powers of delta, theta, alpha, and beta waves between the resting condition and sweet almond oil inhalation. Repeated administration of sweet almond oil also showed no significant changes in all brain wave powers (Table 2).

Table 1: Demographic data for the sweet almond oil inhaling participants

Parameters	**Number**	**Mean**	**SD**
Age (years)	25	20.20	0.41
Height (cm)	25	1.60	0.06
Weight (kg)	25	51.96	4.11
Body Mass Index (kg/m^2)	25	20.31	1.25

EEG data

The brain wave parameters during resting, the first period of sweet almond oil inhalation (SO1), and the second period of sweet almond oil inhalation (SO2) are shown in Table 2.

Table 2: Brain wave power values for the resting condition (the closed eye recording) and two periods of sweet almond oil inhalation

Area	**Delta Power (μV^2)**							
	Resting		**SO1**		**SO2**		**p-value resting and SO1**	**p-value SO1 and SO2**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Left anterior	6.55	2.09	6.32	1.32	6.58	1.76	0.543	0.372
Right anterior	6.73	1.58	6.64	1.78	6.82	2.24	0.780	0.552
Central area	10.79	3.21	10.11	2.72	10.08	3.04	0.181	0.926
Left posterior	4.99	1.84	4.70	1.33	5.01	1.70	0.344	0.291
Right posterior	4.68	1.47	4.39	0.86	4.50	1.17	0.312	0.521
Area	**Theta Power (μV^2)**							
	Resting		**SO1**		**SO2**		**p-value resting and SO1**	**p-value SO1 and SO2**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Left anterior	4.13	1.31	4.04	1.20	4.04	1.12	0.483	0.978
Right anterior	4.50	1.29	4.43	1.31	4.46	1.56	0.591	0.817
Central area	8.99	3.23	8.38	2.68	8.65	2.30	0.224	0.240
Left posterior	4.01	2.24	3.77	1.66	4.01	1.86	0.334	0.276

Right posterior	3.69	1.86	3.50	1.29	3.61	1.46	0.407	0.659
Area	**Alpha Power (μV^2)**							
	Resting		**SO1**		**SO2**		**p-value resting and SO1**	**p-value SO1 and SO2**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Left anterior	5.58	3.76	5.61	4.07	6.07	4.06	0.934	0.245
Right anterior	6.76	4.87	6.63	4.68	6.90	4.69	0.787	0.580
Central area	7.05	3.19	6.88	3.49	7.18	2.97	0.626	0.390
Left posterior	6.10	4.05	6.41	3.99	5.56	3.45	0.427	0.599
Right posterior	5.47	2.30	5.53	2.23	6.06	1.86	0.840	0.135
Area	**Beta Power (μV^2)**							
	Resting		**SO1**		**SO2**		**p-value resting and SO1**	**p-value SO1 and SO2**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**		
Left anterior	3.47	0.99	3.43	0.89	3.51	0.81	0.873	0.556
Right anterior	3.96	1.37	3.86	1.32	4.01	1.32	0.447	0.255
Central area	5.01	1.27	4.97	1.05	5.04	1.08	0.770	0.620
Left posterior	4.17	1.48	4.34	1.44	4.62	1.53	0.375	0.175
Right posterior	4.53	1.36	4.71	1.44	4.67	1.39	0.343	0.750

** Significant difference, p-value < 0.05, SO1, SO2 - the first and second periods of sweet almond oil inhalation*

DISCUSSION

Two inhalations of sweet almond oil were compared, and based on the EEG recordings, no significant changes in brain wave activities were found. Essential oils, including sweet almond oil, contain fragrance molecules that stimulate olfactory receptor cells in the nose; then, these oils open channels, leading olfactory sensory neurons to send electrical signals to the brain *via* the olfactory bulb and higher olfactory cortex[15]. An EEG recording is a non-invasive and painless technique for understanding the psychophysiological activities induced by essential oils. During neural excitations, EEG recordings can measure the changes in electric potentials *via* electrodes placed on the scalp. The signals reflecting brain electrical activity consist of different characteristic waves. Furthermore, various regions of the brain emit different brain wave frequencies simultaneously[25]. The A-B experimental design is a within-subjects study design, meaning that each individual session consisted of two trails. This design was chosen because, with olfactory stimulation, the time course of the stimulatory effects is unknown, which might make results obtained from other designs, such as A-B-A, difficult to interpret. As the time of return to baseline functioning after olfactory stimulation may vary to an unknown degree, the use of an A–B design with experimental and corresponding control groups seemed the most promising and economic approach[26]. The A-B protocol in this research has been set by our group to evaluate and classify the effective potential of the essential oils commonly used in Thailand.

The results in this study were consistent with previous studies that used sweet almond oil. For instance, Sayorwan *et al.* (2013) investigated brain wave activities among 20 healthy participants through an A-B experimental design. The procedure was divided into 3 sessions of 7 min each. Firstly, resting (baseline) EEG recordings were done separately with both eye-closed and eye-opened sessions. Following these sessions, the participants were exposed to sweet almond oil and then to 10% v/v rosemary oil (in sweet almond oil) at 5-minute intervals. The rosemary oil inhalation decreased the powers of alpha waves of the left anterior, right anterior, left posterior, and right posterior areas and the central brain regions. Furthermore, rosemary oil inhalation increased the powers of beta waves of the left and right anterior regions significantly when compared with sweet almond oil. Their results therefore confirmed the stimulatory effects of rosemary oil. Moreover, sweet almond oil did not change any brain wave powers when compared with the resting condition. On the contrary, similar experiments on the autonomic nervous system and mood states found that sweet almond oil significantly decreased heart rate and increased drowsiness compared to the resting condition. However, the comparison between sweet almond oil and rosemary oil inhalation showed significant increases in heart rate, blood pressure, and respiratory rate as well as a decrease in skin temperature. After exposure to rosemary oil, the participants felt fresher, more active, and less drowsy when compared with sweet almond oil[16].

The A-B design studies on brain wave activities demonstrated the relaxing effect of lavender oil, the stimulating effects of rosemary oil and jasmine oil, and the relaxed concentration effect of citronella oil (the increase of alpha and beta brain powers) [16-17,27-28]. Additionally, the inhalation of fingered citron peel oil was found to have a stimulating effect on the autonomic nervous system and on emotional states. Both blood pressure and respiratory rates showed a significant increase upon exposure to fingered citron essential oil (10% v/v in sweet almond oil) *versus* sweet almond oil[29].

Ferdenzi *et al.* (2014) found that repeated exposure to odors can affect their pleasantness[30]. Chamine and Oken evaluated the effects of lavender aroma, which is commonly used for stress reduction, and concluded that aroma hedonics (pleasantness and intensity) contributed to a beneficial effect on working memory and physiological functions[31]. This research revealed the inertness of sweet almond oil, which was administered repeatedly as a placebo and also as an essential oil diluent in an A-B design. Another recent study verified the effects of inhaled *Limnophila aromatica* essential oil on brain wave activities and emotional states in healthy volunteers, using sweet almond oil as a control. The results likewise showed that sweet almond oil did not cause any significant changes in the band powers of delta, theta, alpha, and beta waves in all brain regions[32].

CONCLUSION

In this study, the A-B experimental design was performed. The research was conducted to test whether twice inhaling sweet almond oil affected EEG recordings. The study showed that the second inhalation of sweet almond oil did not cause any significant changes in brain wave activities among healthy participants.

RECOMMENDATION

Sweet almond oil can be used as a base oil in essential oil preparation for brain wave activity research.

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