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Effect of olfactory stimulation of isomeric aroma compounds, (+)-limonene and terpinolene on human electroencephalographic activity

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ABSTRACT

Introduction: The present study was undertaken to evaluate the olfactory stimulation of (+)-limonene and terpinolene on human electroencephalographic (EEG) activity in order to understand their action on brain function by isomer compounds.
Methods: The effect of inhalation of isomers on EEG activity was evaluated by the measurement of EEG power spectrum in 18 healthy participants. EEGs were recorded using QEEG-8 system from 8 grounding electrodes according to the International 10–20 System.
Results: The results indicated that the relative high beta (RHB) activity increased significantly in the right temporal region (from 0.1250 to 0.1429 μV, $p < 0.05$) during the inhalation of (+)-limonene. Whereas, the relative mid beta (RMB) activity decreased significantly (from 0.0646 to 0.0561 μV, $p < 0.05$) and the relative fast alpha (RFA) activity increased significantly in the right prefrontal region (from 5.6215 to 7.3336 μV, $p < 0.05$) during the inhalation of terpinolene. According to gender variation, significant changes of absolute and relative beta activities were observed in men than women during the inhalation of both the compounds. Women responded well to both the compounds by a significant increase of absolute fast alpha activity. When compared with (+)-limonene, terpinolene exhibited positive effect by reducing the tension and increasing the relaxation and stabilization states of brain function.
Conclusion: The isomers produce different states of brain function as affect the different regions of brain. Further, the EEG activity of isomers on human brain is highly related to the structural arrangement of individual compounds and gender difference.

1. Introduction

Essential oils have been used for thousands of years to aid various psychophysiological disorders such as depression, anxiety and cognitive disorders. The inhalation of essential oils can communicate signals to the olfactory system and produce psychological alterations in humans [1]. Fragrance inhalation of essential oils of lavender, peppermint, rosemary and clary-sage significantly reduced the anxiety and stress states of brain function [2]. Essential oils are natural, complex mixtures of various aroma compounds, mainly composed of terpenes and their oxygenated derivatives [3]. Individual chemical components of essential oils (mono- and sesquiterpene compounds) also possess various psychophysiological properties. These components contribute to the beneficial or adverse effects on biological systems. Previous studies have reported the isomers of aromatic compounds such as carvone, linalool and limonene possess different fragrance qualities and intensities for humans [4–6]. Interestingly, in the case of enantiomers of 3-methylthiobutanal, one has a specific aroma while another one is odorless [7]. Due to fragrance differences between the isomer compounds, their medicinal and other biological properties also different [8].

(+)-Limonene [(*R*)-(+)-limonene (Isopropenyl-1-methyl-1-cyclohexene)], a monocyclic monoterpene, is the most widespread terpene with possessing strong smell of oranges and occurs in more than 300 plants [9]. (+)-Limonene is present high levels in orange oil (more than 90%) followed by grapefruit oil (90%), lemon oil (70%) and celery oil (60%) etc. [10]. Major industrial applications of this compound is as an additive in food products, cosmetics and perfumes for flavor and fragrance [11]. It has been identified as a non-toxic agent with the potential for cancer chemotherapy. Several studies have reported that (+)-limonene prevents the formation of chemically induced tumors [12]. Lima et al. [13] studied that (+)-limonene has an anti-anxietic effect on mice model. Heuberger et al. [14] reported the inhalation of

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enantiomers of limonene and carvone highly influenced on the human autonomic nervous system parameters and mental conditions. Terpinolene (4-isopropylidene-1-methylcyclohexene) is a structural isomer of (+)-limonene, a monocyclic monoterpene compound containing a cyclohexene ring with having the smell of pine fragrance. It is a main constituent of essential oils of various pine species, as well as other plants such as *Citrus* species, *Manilla elemi*,*Nectranda elaiophora* and *Dacrydium colensoi* [9]. It has been mainly used as a food flavoring additive in artificial essential oils, fruits, citrus, ice cream, non-alcoholic beverages, candy and baked goods [15]. Ito and Ito [16] studied the sedative effect of terpinolene and its structure–activity relationship in a mouse model. Further, the authors compared terpinolene analog activities and suggested that a double bond in the side-chain or pi bonds in the six-membered ring play significant roles in the sedative effect.

(+)-Limonene and terpinolene have the same molecular formula ($C_{10}H_{16}$) but the arrangement of atoms and their fragrances are different. Further, there is no study in relation to the effect of these isomer compounds on EEG activity of human brain. Hence, the present study was carried out to determine the effect of olfactory stimulation of isomeric compounds, (+)-limonene and terpinolene on human EEG activity to assess whether their action on brain function is the same or different.

2. Methods

2.1. Materials

(+)-Limonene (CAS No. 5989-27-5) and terpinolene (CAS No. 586-62-9) were purchased from Sigma (St. Louis, MO, USA). The study was approved by the ethics committee from the Kangwon National University Hospital, Chuncheon, Republic of Korea (IRB File No. KWNUH 2013-11-008-003). All participants provided written informed consent.

2.2. Subjects

Eighteen right-handed healthy volunteers (9 men and 9 women) aged 20–30 years participated in this study. None of the subjects had olfactory diseases, smoked or abused drugs. All subjects gave their informed consent before participation.

2.3. EEG recordings

EEGs were recorded using QEEG-8 system (LXE3208, LAXTHA Inc., Daejeon, Korea) from 8 grounding electrodes placed on the scalp at left prefrontal (Fp1), right prefrontal (Fp2), left frontal (F3), right frontal (F4), left temporal (T3), right temporal (T4), left parietal (P3) and right parietal (P4) according to the International 10–20 System. All electrodes were referenced to the ipsilateral earlobe electrode.

2.4. Fragrance administration

(+)-Limonene and terpinolene (10%) were dissolved in ethanol (90%) and used as fragrance stimuli. These stimuli were separately presented to the subjects in a randomized sequence. EEG measurement sites maintain a constant temperature (23 °C) and humidity (50%) to 32.5 m^2 size of the laboratory. The subjects were instructed to sit quietly, close their eyes and to breathe normally during the measurement. The fragrance stimuli were separately dipped in a filter paper (1 cm^2) then placed about 3 cm in front of the subject's nose. EEG was recorded for 30 s before and during fragrance exposure.

2.5. Data analysis

The mean power values [microvolt (μV)] were calculated for 25 EEG analysis indicators (Table 1). The t-mapping of EEG waves of brain was constructed by using Telescan software package (LXSMD61, LAXTHA Inc., Daejeon, Korea). The SPSS statistical package 18 (SPSS, Inc., Chicago, IL, USA) was used for data analysis on EEG activity before and during the exposure of (+)-limonene/terpinolene by a paired *t*-test based on the EEG power spectrum values.

3. Results

In the present study, olfactory stimulation analysis with the inhalation of (+)-limonene and terpinolene at a constant

Table 1
EEG power spectrum indicators used in this study.

S. No.	Analysis indicators	The full name of the EEG power spectrum indicators	Wavelength range (Hz)
1	AT	Absolute theta	4–8
2	AA	Absolute alpha	8–13
3	AB	Absolute beta	13–30
4	AG	Absolute gamma	30–50
5	ASA	Absolute slow alpha	8–11
6	AFA	Absolute fast alpha	11–13
7	ALB	Absolute low beta	12–15
8	AMB	Absolute mid beta	15–20
9	AHB	Absolute high beta	20–30
10	RT	Relative theta	(4–8)/(4–50)
11	RA	Relative alpha	(8–13)/(4–50)
12	RB	Relative beta	(13–30)/(4–50)
13	RG	Relative gamma	(30–50)/(4–50)
14	RSA	Relative slow alpha	(8–11)/(4–50)
15	RFA	Relative fast alpha	(11–13)/(4–50)
16	RLB	Relative low beta	(12–15)/(4–50)
17	RMB	Relative mid beta	(15–20)/(4–50)
18	RHB	Relative high beta	(20–30)/(4–50)
19	RST	Ratio of SMR to theta	(12–15)/(4–8)
20	RMT	Ratio of mid beta to theta	(15–20)/(4–8)
21	RSMT	Ratio of SMR–mid beta to theta	(12–20)/(4–8)
22	RAHB	Ratio of alpha to high beta	(8–13)/(20–30)
23	SEF50	Spectral edge frequency 50%	4–50
24	SEF60	Spectral edge frequency 90%	4–50
25	ASEF	Spectral edge frequency 50% of alpha	8–13