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Effect of Essential Oil from San-Jo-In (*Zizyphus jujuba* Mill. seeds) on Human Electroencephalographic Activity

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Essential oils have been used to treat psychophysiological disorders, but their effects on human electroencephalographic (EEG) activity have not been thoroughly investigated. We evaluated the effects of essential oil of *Zizyphus jujuba* seeds, a Korean folk medicine known as San-Jo-In, on human EEG activity. For this purpose, essential oil was extracted from San-Jo-In by the supercritical carbon dioxide extraction method. The effect of its inhalation on EEG activity was evaluated by measuring the EEG power spectrum (25 indices) in 20 healthy participants. The results of the EEG power spectrum indicated that the values of the theta wave decreased significantly ($p<0.05$) in the left (from 17.277 to 13.854 μV) and right parietal (from 15.324 to 13.020 μV) regions compared to the other regions. During the inhalation of San-Jo-In oil, the EEG spectrum values of fast alpha, relative gamma, and spectral edge frequency increased 50% compared to those before inhalation. The values of the fast alpha wave increased significantly ($p<0.05$) in the left prefrontal (from 0.063 to 0.085 μV), right prefrontal (from 0.064 to 0.085 μV), and left frontal (from 0.073 to 0.100 μV) regions following inhalation of the San-Jo-In essential oil. The changes in the EEG activities following inhalation of San-Jo-In suggest that the oil can improve psychological well-being by increasing attention and relaxation.

Key words : Aromatherapy, electroencephalogram, essential oil, San-Jo-In, *Zizyphus jujuba*

Introduction

The therapeutic use of aromatic plants has a long tradition and their pharmaceutical properties are mostly ascribed to its essential oils. Essential oils are natural, complex, multi-component systems mainly composed of terpenes in addition to some other non-terpene components [3]. Aromatherapy is the therapeutic use of essential oils from plants. One of the methods used in the aromatherapy is release of odor to a particular environment. The sense of smell plays an important role in the psychophysiological function of human beings. A number of studies have found that olfactory stimulation by inhalation of essential oils produces immediate changes in physiological parameters such as blood pressure, muscle tension, pupil dilation, skin temperature, pulse rate and brain activity [8, 9, 17]. Moreover, fragrance molecules have the advantage of acting through olfactory pathways without decomposition in the digestive organs [25]. Previous studies have indicated that the fragrance molecules absorbed by inhalation, are able to cross the blood-brain barrier and interact with receptors in the central nervous system [5, 22]. In this context, investigations of essential oils on EEG activity of human brain have also become important and worthwhile for the treatment of physiological disorders.

Zizyphus jujuba Mill. is a perennial tree in the Rhamnaceae family and has a wide natural distribution in tropical and subtropical parts of the world [21]. The jujube fruits are edible and have been used in Korean folk medicine as San-Jo-In and in Chinese folk medicine as Suan-Zao-Ren or Suan-Zao-He with high sugar content and high levels of vitamin A, B and C complexes, phosphorus and calcium [24]. This plant possesses various pharmacological properties such as liver problems, insomnia and anxiety [13, 28]. Kim [14] reported that the jujube seeds improve the blood glucose level and lipid profile in serum of dietary hyperlipidemic rats.

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Al-Reza et al. [1, 2] reported that the essential oil of jujube seed registered potent anti-oxidant, anti-listerial and anti-inflammatory activities. However, there is no study related to the effects of its essential oil on EEG activity of human brain. Hence, the present study was carried out to evaluate the EEG activity of human brain with respect to fragrance inhalation of essential oil of *Z. jujuba* seeds.

Materials and Methods

Plant material

The dried San-Jo-In were purchased from BN Herb Inc., Pyeongchang, Korea. The plant material was dried at room temperature, ground to a powder using a blender and stored at -20°C prior to use.

Essential oil

Extraction of essential oil from San-Jo-In was experimentally determined using supercritical fluid extraction equipment (ILSHIN Autoclave Co. Ltd., Korea) at the temperature of 60°C and pressure of 400 bar with carbon dioxide flow rate of 30 ml/min.

Effect of inhalation of San-Jo-In essential oil on EEG activity

Participants

We investigated the effects of essential oil from San-Jo-In on EEG findings in twenty right-handed healthy volunteers (10 men and 10 women) aged 20-30 years. None of the participants had olfactory diseases, smoked or abused drugs. The purpose and schedule of the experiments were explained to participants and received written informed consent before participation.

EEG recordings

EEGs signals 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.

Fragrance administration

The essential oil of San-Jo-In was used as the fragrance stimulus. The stimulus was 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 stimulus was dipped in a filer paper (1 cm^2) then placed about 3 cm in front of the subject's nose. EEG was recorded before and during the fragrance exposure for 30 seconds.

Data analysis

The mean power values (μV) calculated for 25 EEG analysis indicators (Table 1). The t-mapping of EEG waves was constructed by using Telescan software package (LXSMD61, LAXTHA Inc., Daejeon, Korea). The SPSS statistical package 18 (SPSS, Inc., Chicago, IL, USA) used for data analysis on EEG activity before and during the exposure of the essential oil of San-Jo-In by a paired t-test based on the EEG power spectrum values.

Results and Discussion

Aromatherapy is a kind of alternative medicine that uses essential oils for psychological and physical wellbeing of humans through fragrance inhalation. Fragrance inhalation of essential oils has a substantial role in controlling the functions of central nervous system. In Chinese folk medicine, the fragrance inhalation of essential oils of *Storax pill* and *Acorus gramineus* rhizome are used for the treatment of epilepsy [15, 16]. In an animal study, Koo et al. [15] stated that the inhibitory effect on the central nervous system by essentials oils through the gamma-aminobutyric acid-ergic neuromodulation system. Yamada et al. [26] studied that the fragrance inhalation of lavender essential oil is effective in reducing tension. Fragrance inhalation of essential oils of lavender, peppermint, rosemary and clary-sage were significantly reduced the anxiety and stress states of brain function [11].

The objective of this study was to understand the effect of inhalation of San-Jo-In essential oil on the activities of human brain using EEG. The EEG power spectrum values recorded for 8 electrode sites (Fp1, Fp2, F3, F4, T3, T4, P3 and P4) of the international 10-20 system. In the present study, a total of 25 EEG indices analyzed for 8 electrode sites. Among the 25 indices analyzed, significant changes (p<0.05) observed in the EEG power spectrum values with

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	AFA	Absolute slow alpha	8~11
6	ASA	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	RFA	Relative slow alpha	(8~11) / (4~50)
15	RSA	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

increase of absolute theta, relative gamma, relative fast alpha and decrease of spectral edge frequency 50% during inhalation when compared to before inhalation, but had no effect on other EEG indices. The changes in the values of EEG power spectrum of human brain before and during the inhalation of San-Jo-In oil are presented in Table 2-5. Figure 1 also illustrates the differences in the t-mapping of absolute theta, relative gamma, relative fast alpha and spectral edge frequency 50% waves before and during the inhalation of San-Jo-In oil.

The values of theta waves decreased significantly ($p<0.05$) in the regions of left (from 17.277 to 13.854 μV) and right parietal (from 15.324 to 13.020 μV), however, there were no significant effects in the frontal and temporal regions (Table 2). On the other hand, the values of fast alpha waves increased significantly ($p<0.05$) in the left prefrontal (from

Table 2. Changes of absolute theta power spectrum values before and during the inhalation of essential oil of San-Jo-In (*Z. jujube* seeds)

Site	Absolute theta values (μV)		t-test	*p* value
	Before	During		
Fp1 - Left prefrontal	13.871	13.067	0.880	0.390
Fp2 - Right prefrontal	12.944	12.514	0.329	0.746
F3 - Left frontal	19.036	17.125	1.334	0.198
F4 - Right frontal	18.403	17.705	0.338	0.739
T3 - Left temporal	11.422	10.413	1.232	0.233
T4 - Right temporal	6.358	6.159	0.261	0.797
P3 - Left parietal	17.277	13.854	2.750	0.013*
P4 - Right parietal	15.324	13.020	2.729	0.013*

*Significant difference ($p<0.05$)

Table 3. Changes of relative gamma power spectrum values before and during the inhalation of essential oil of San-Jo-In (*Z. jujube* seeds)

Site	Relative gamma values (μV)		t-test	*p* value
	Before	During		
Fp1 - Left prefrontal	0.085	0.091	-1.053	0.306
Fp2 - Right prefrontal	0.091	0.093	-0.425	0.676
F3 - Left frontal	0.040	0.049	-2.569	0.019*
F4 - Right frontal	0.036	0.041	-1.703	0.105
T3 - Left temporal	0.122	0.130	-0.632	0.535
T4 - Right temporal	0.128	0.134	-0.399	0.695
P3 - Left parietal	0.044	0.049	-1.002	0.329
P4 - Right parietal	0.041	0.041	-0.198	0.845

*Significant difference ($p<0.05$)

Table 4. Changes of relative fast alpha power spectrum values before and during the inhalation of essential oil of San-Jo-In (*Z. jujube* seeds)

Site	Relative fast alpha values (μV)		t-test	*p* value
	Before	During		
Fp1 - Left prefrontal	0.063	0.085	-2.167	0.043*
Fp2 - Right prefrontal	0.064	0.085	-2.133	0.046*
F3 - Left frontal	0.073	0.100	-2.151	0.045*
F4 - Right frontal	0.071	0.095	-1.949	0.066
T3 - Left temporal	0.072	0.088	-2.398	0.027
T4 - Right temporal	0.070	0.080	-1.165	0.258
P3 - Left parietal	0.115	0.125	-0.761	0.456
P4 - Right parietal	0.109	0.120	-0.872	0.394

*Significant difference ($p<0.05$)

Table 5. Changes of spectral edge frequency 50% power spectrum values before and during the inhalation of essential oil of San-Jo-In (*Z. jujube* seeds)

Site	Spectral edge frequency 50% values (μV)		t-test	*p* value
	Before	During		
Fp1 - Left prefrontal	10.447	10.519	-0.520	0.609
Fp2 - Right prefrontal	11.059	11.153	-0.760	0.457
F3 - Left frontal	9.619	9.884	-2.332	0.031*
F4 - Right frontal	9.619	9.709	-0.716	0.482
T3 - Left temporal	12.950	13.316	-0.694	0.496
T4 - Right temporal	12.650	12.797	-0.166	0.870
P3 - Left parietal	9.988	10.106	-1.109	0.281
P4 - Right parietal	9.888	10.081	-1.591	0.128

*Significant difference ($p<0.05$)

0.063 to 0.085 μV), right prefrontal (from 0.064 to 0.085 μV) and left frontal (from 0.073 to 0.100 μV) regions due to the inhalation of essential oil of San-Jo-In (Table 4). Further, the values of relative gamma waves (from 0.040 to 0.049 μV) and spectral edge frequency 50% (from 9.619 to 9.884 μV) increased significantly in the region of left frontal during inhalation of San-Jo-In oil when compared with before inhalation (Table 3 and 5). Based on the results, the essential oil of San-Jo-In highly alters the EEG power spectrum values in the regions of parietal and frontal regions and did not significantly alter the values in the temporal regions. Craig [7] suggested that the front lobes of human brain are important in the regulation of emotional feelings. Especially, left frontal region is involved in the management of arousal

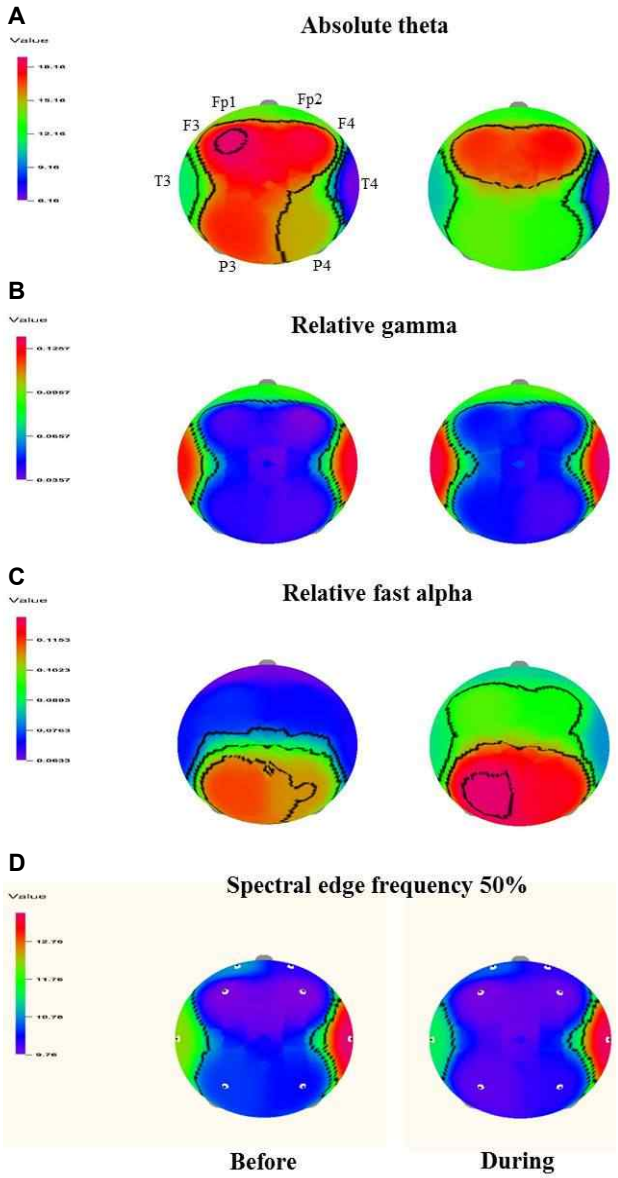

Fig. 1. t-Mapping of absolute theta (A), relative gamma (B), relative fast alpha (C) and spectral edge frequency 50% (D) before and during the inhalation of essential oil of *Z. jujuba* seeds. Fp1, left prefrontal; Fp2, right prefrontal; F3, left frontal; F4, right frontal; T3, left temporal; T4, right temporal; P3, left parietal; P4, right parietal.

and stress. These findings indicate that aromatic compounds emitted from the essential oil of San-Jo-In may influence in the changes of EEG power spectrum values of human brain.

Similar to our report, Iijima et al. [12] studied that the inhalation of agarwood incense significantly increased the fast alpha activity. Previous studies demonstrated that the effects of fragrance inhalation of lavender oil increased alpha activity and these changes afforded the relaxation of brain function [8, 17]. Inhalation of essential oils of eucalyptus, lavender and spiced oil have been found to possess potent and positive effects on EEG activity [10]. In contrary to our results, Masumoto et al. [18] reported that the inhalation of aroma of spearmint oil significantly decreased alpha and increased theta activities. Then the authors suggested that the spearmint oil induced arousal effects. Some authors have denoted that alpha and theta waves are related to hypnagogic state, and are modulated by behavior and brain state [6]. Matsubara et al. [19] studied that the essential oil of *Abies sibirica* leaves decreases arousal level by significant increase of theta power at the recovery period compared with at working period. Borbely et al. [4] reported that the values of alpha waves gradually decrease and theta waves gradually increase, when the eyes are closed. From the results, it could be stated that the essential oil of San-Jo-In has potential to prevent the mental health problems in related to anxiety.

Al-Reza et al. [1] studied that the essential oil composition of San-Jo-In contains 23 compounds, representing 91.59% of the total oil and the major compounds are eugenol (48.3%), isoeugenol (11.83%), caryophyllene (9.16%), eucalyptol (3.27 %), and caryophyllene oxide (3.14%). The major component, eugenol also found high concentration in the essential oils of many aromatic plants such as *Ocimim basilicum*, *O. tenuiflorum* and *Eugenia caryophyllata* [20, 23, 27]. In the present investigation, the effect of San-Jo-In on EEG activity of human brain is may be attributed to the major component, eugenol and other aromatic components.

In conclusion, it is suggested that the essential oil of San-Jo-In are able to alter the EEG activity and might be contribute to the positive effects on mood and cognitive functions of brain. Further studies are warranted to understand the actual mechanism behind the action of essential oils on EEG activity of human brain.

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초록 : 산조인(*Zizyphus jujuba* Mill.) 에센셜오일 흡입이 인간의 뇌파에 미치는 영향

조해미[1] · 유병선[1] · 칸다사미손드하라라잔[1] · 정지욱[2] · 주진우[3] · 김성문[1]*
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에센셜오일은 신경생리학적 장애의 치료에 오랜동안 사용되었지만 아직까지 그 효능이 과학적으로 구명되지 않았다. 저자들은 한의학에서 산조인으로 알려져 있는 *Zizyphus jujuba* 종자의 에센션오일이 인간의 뇌파에 미치는 영향을 연구하였다. 초임계이산화탄소추출법을 이용하여 산조인으로부터 에센셜오일을 얻고, 20명의 피험자를 대상으로 EEG 파워 스펙트럼을 측정하였다. 산조인 에센셜오일 흡입 전과 흡입 중 다른 부위와 비교하여 theta파가 좌측 두정엽 부위(17.277→13.854 μV)와 우측 두정엽 부위(15.324 →13.020 μV)에서 통계학적으로 차이를 나타내었다(p<0.05). 산조인 정유 흡입으로 fast alpha 파, relative gamma 파, spectral edge frequency 50% 지수가 증가하였는데, 이들 지수 중 fast alpha 파는 좌측 전전두엽 부위(0.063→0.085 μV), 우측 전전두엽 부위(0.064→0.085 μV), 그리고 좌측 두정엽 부위(0.073→0.100 μV)에서 통계학적으로 차이를 나타내었다. 본 연구의 결과는 산조인 에센셜오일 흡입에 의한 EEG 변화는 두뇌기능 중 각성과 진정상태를 증가시켜 인간의 심리학적인 조건을 향상시킨다는 것을 추론하게 한다.