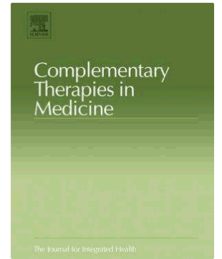


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# Modulatory effects of aromatherapy massage intervention on electroencephalogram, psychological assessments, salivary cortisol and plasma brain-derived neurotrophic factor



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## KEYWORDS

Aromatherapy  
massage;  
Stress;  
BDNF;  
EEG;  
Alpha wave;  
Cortisol

## Summary

**Objectives:** Aromatherapy massage is commonly used for the stress management of healthy individuals, and also has been often employed as a therapeutic use for pain control and alleviating psychological distress, such as anxiety and depression, in oncological palliative care patients. However, the exact biological basis of aromatherapy massage is poorly understood. Therefore, we evaluated here the effects of aromatherapy massage interventions on multiple neurobiological indices such as quantitative psychological assessments, electroencephalogram (EEG) power spectrum pattern, salivary cortisol and plasma brain-derived neurotrophic factor (BDNF) levels.

**Design:** A control group without treatment ( $n=12$ ) and aromatherapy massage group ( $n=13$ ) were randomly recruited. They were all females whose children were diagnosed as attention deficit hyperactivity disorder and followed up in the Department of Psychiatry, Jeju National University Hospital. Participants were treated with aromatherapy massage for 40 min twice per week for 4 weeks (8 interventions).

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**Results:** A 4-week-aromatherapy massage program significantly improved all psychological assessment scores in the Stat-Trait Anxiety Index, Beck Depression Inventory and Short Form of Psychosocial Well-being Index. Interestingly, plasma BDNF levels were significantly increased after a 4 week-aromatherapy massage program. Alpha-brain wave activities were significantly enhanced and delta wave activities were markedly reduced following the one-time aromatherapy massage treatment, as shown in the meditation and neurofeedback training. In addition, salivary cortisol levels were significantly reduced following the one-time aromatherapy massage treatment.

**Conclusions:** These results suggest that aromatherapy massage could exert significant influences on multiple neurobiological indices such as EEG pattern, salivary cortisol and plasma BDNF levels as well as psychological assessments.

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## Introduction

The overload of stress causes several physical and emotional distress and stress-related illnesses, depending on the individual's stress vulnerability. Therefore, several complimentary techniques such as meditation, muscle relaxation, and biofeedback have been developed for effective stress management.<sup>1</sup> Aromatherapy massage is commonly used for the stress management of healthy individuals, and also has been employed for therapeutic use of pain control and alleviating psychological distress such as anxiety and depression in cancer patients<sup>2</sup> and palliative care.<sup>3</sup>

However, the exact biological basis of aromatherapy massage is poorly understood. Very few have been published regarding biological outcome measures following these kinds of treatments. Aromatherapy massage induced a differential alteration of lymphocyte subset.<sup>4</sup> Massage intervention affected brain development and the maturation of brain electrical activity such as visual evoked potentials (VEPs) in human infants and in rat pups. In addition, higher levels of blood insulin-like growth factor (IGF) were found in massaged infants and similarly, IGF expression levels were increased in the cortex of massaged rat pups.<sup>5</sup>

Electroencephalography (EEG) is used as a neurophysiological evaluation tool to reflect the brain state or brain activity. EEG rhythms are categorized by their frequency range. Traditionally, four major EEG rhythms are used. These are delta waves (<4 Hz), theta waves (4–8 Hz), alpha waves (8–13 Hz) and beta waves (greater than about 13 Hz).<sup>6</sup> Several EEG studies have demonstrated significant alterations in spectral band frequencies during meditation. Brain activities of alpha rhythm were generally enhanced during meditation and theta rhythm activities were prominent in more experienced meditators.<sup>7</sup> Moreover, alpha waves in EEG have been the main target of training-induced alterations by operant conditioning in the neurofeedback. Several studies demonstrated that enhanced EEG alpha power by neurofeedback training improved the cognitive task performance.<sup>8</sup>

Therefore, we evaluated here the effects of aromatherapy massage interventions on multiple neurobiological indices such as EEG pattern, salivary cortisol and plasma BDNF levels as well as psychological assessments.

## Methods

### Participants

A total of 25 subjects aged 34–48 years volunteered to participate in the present study. They were randomly assigned to either the aromatherapy massage group ( $n=13$ ) or control group ( $n=12$ ). They were all females whose children were diagnosed as attention deficit hyperactivity disorder and followed up in the Department of Psychiatry, Jeju National University Hospital. This study was approved by the Institutional Review Board of the Jeju National University Hospital before obtaining written consents from the participants.

### Aromatherapy massage program

All participants in the treatment group were administered with aromatherapy massage for 40 min twice per week for 4 weeks (eight times in total). The interventions were conducted by trained therapists according to the following protocol. After bath, each subject was applied with 20 ml of Jojoba oil containing mixed essential oils of *Lavandula angustifolia* (lavender, 2%, ShirleyPrice Aromatherapy Co., UK) and *Pelargonium graveolens* (geranium, 2%, ShirleyPrice Aromatherapy Co., UK). The treatment dose was based on the previous reports.<sup>4</sup> Either lavender or geranium are currently in use as a aromatherapy agent to relieve anxiety, stress and depression.<sup>9</sup> The effleurage, friction, petrissage and vibration were included in the massage treatment. The same procedures were applied in all treatment group participants on the neck, shoulder, arms, back and legs at a moderate pressure, according to the same protocol.

### EEG recording and power spectrum analysis

The participants were seated with eyes closed in a comfortable armchair for EEG recording. EEG data were acquired for 2 min using the computerized EEG recording system BIOPAC MP36 (BIOPAC systems, USA). The recording electrodes were placed on Fp1 and Fp2 (prefrontal), and on O1 and O2 (occipital) scalp regions according to the international 10–20 electrode system (American Electroencephalographic Society, 1994) using an ECI electrode cap (Electro-Cap International, OH, USA). They were then



connected to the BIOPAC MP36 system with flexible insulated cables. All channels were referenced to the linked earlobes. Electrode impedances were kept below 3 k $\Omega$ . EEG signals were acquired at a sampling rate of 1000 Hz, filtered with a band-pass filter of 0.5–35 Hz and analyzed using the BrainMAP-3D 2.0 program (LAXTHA Inc, Korea). Power spectrum analysis was performed to investigate the individual brain wave activities. The individual electrical power data ( $\mu$ V) of frequencies-based brain waves were exported to the BrainMAP-3D 2.0 program. The relative power of each of the four spectral bands was expressed as mean  $\pm$  SEM percentage (%) of the total spectral power within the 0.5–45 Hz window, as previously reported.<sup>10,11</sup>

### Measurement of plasma BDNF

Plasma brain-derived neurotrophic factor (BDNF) concentration was determined using enzyme immunoassay (EIA) according to the manufacturer's protocol (ChemiKine BDNF Sandwich EIA Kit, Chemicon, USA).

### Measurement of cortisol

Cortisol concentration in the either salivary fluid or the plasma was determined using competitive EIA according to the manufacturer's protocol (Cortisol EIA kit, ALPCO Diagnostics, USA).

### Blood sampling

All participants including the control and therapy groups visited the Jeju National University Hospital for blood sampling at 3 p.m. on the same day before and after the 4 week aromatherapy massage program. Blood sampling was designed to investigate the effect of the 4 week aromatherapy massage program on plasma BDNF and cortisol levels.

### Salivary sampling

The salivary samples were taken immediately before and after one time-aromatherapy massage treatment in order to investigate the effect of the one-time treatment on salivary cortisol levels. After rinsing the mouth, 2 ml of salivary fluid was collected and centrifuged, and the supernatant was stored frozen at  $-20^{\circ}\text{C}$  before use.

### State-Trait Anxiety Index (STAI)

The state-trait anxiety inventory (STAI)-KYZ was used to measure the subjects' degree of anxiety,<sup>12</sup> which is a widely used, validated measure of anxiety levels. The internal consistency (Cronbach's alpha coefficient) of STAI-KYZ was 0.97 in this study.

### Beck Depression Inventory (BDI)

The Beck Depression Inventory (BDI)<sup>13</sup> is a widely used, validated measure of depression levels. The internal consistency (Cronbach's alpha coefficient) of BDI was 0.94 in this study.

### Short Form of Psychosocial Well-being Index (PWI-SF)

The stress levels of the subjects were measured using a psychosocial well being index-short form (PWI-SF) questionnaire developed by Chang,<sup>14</sup> based on the General Health Questionnaire by Goldberg and his colleagues.<sup>15</sup> The internal consistency (Cronbach's alpha coefficient) of the PWI-SF was 0.95 in this study.

### Statistics

Statistical analyses were conducted using SPSS 14.0 software. Analysis of covariance (ANCOVA) was conducted to analyze group differences between changes in the control and treatment groups after 4-week program. Mann–Whitney *U*-test was employed to analyze the group homogeneity between the control and therapy groups before experiment. Wilcoxon's signed-ranks test was used to analyze pre- and post-differences within group in order to evaluate the treatment effects. Normally distributed variables are presented as means  $\pm$  standard deviation (SD) and non-normally distributed variables are presented as median value and interquartile ranges (IQR; 25th–75th percentile). Statistical significance was accepted for *p* value of  $<0.05$ .

### Results

#### Homogeneity between the therapy and control group

A total of 25 subjects volunteered to participate in the present study. They were all females whose children were diagnosed of attention deficit hyperactivity disorder and followed up in the Department of Psychiatry, Jeju National University Hospital. They were randomly assigned to either the aromatherapy massage group ( $n=13$ ) or control group ( $n=12$ ). There were no statistically significant differences between the therapy and control group in assessment scores in STAI ( $p=0.2$ ), BDI ( $p=0.6$ ) and PWI-SF ( $p=0.4$ ), resting EEG pattern ( $p=0.6$ ), plasma BDNF ( $p=0.2$ ) and cortisol levels ( $p=0.8$ ) based on Mann–Whitney *U*-test, suggesting the homogeneity between the therapy and control group before experiment.

#### Effects of aromatherapy massage on psychological assessments

Pre-post differences in anxiety, depression and stress indices between before and after the 4-week aromatherapy massage program were evaluated in both the therapy and control groups using psychological test batteries such as STAI-KYZ, BDI and PWI-SF. The statistical analyses were conducted using ANCOVA. Anxiety assessment scores in STAI-KYZ were significantly decreased from  $98.2 \pm 23.0$  to  $82.0 \pm 22.3$  ( $p=0.01$ ) after 4-week aromatherapy massage interventions in the therapy group. Both state anxiety scale that evaluates how the subjects feel at the present time and trait anxiety scale that evaluates how the subjects generally feel were significantly reduced from  $47.8 \pm 11.1$  to  $39.6 \pm 10.6$



( $p=0.049$ ) and  $50.3 \pm 12.3$  to  $42.4 \pm 12.3$  ( $p=0.01$ ), respectively. Depression assessment scores in BDI were significantly decreased from  $10.8 \pm 9.9$  to  $6.5 \pm 7.5$  ( $p=0.04$ ) after 4-week interventions. Stress assessment scores in PWI-SF were significantly decreased from  $24.3 \pm 11.2$  to  $17.6 \pm 12.2$  ( $p=0.049$ ) after the 4-week interventions. Taken together, a 4-week aromatherapy massage program significantly improved all psychological assessment scores in STAI-KYZ, BDI and PWI-SF (Table 1).

### Effects of aromatherapy massage on EEG power spectrum pattern

Individual brain rhythms of alpha, beta, theta and delta waves were investigated using the power spectrum analysis. Mean activities of alpha waves from Fp1, Fp2, O1 and O2 electrodes were significantly enhanced from 17.0 (12.1–20.7)% to 25.4 (21.2–30.9)% ( $p=0.02$ ) and the delta activities were significantly reduced from 68.8 (61.3–77.0)% to 54.8 (48.6–68.5)% ( $p=0.02$ ) based on Wilcoxon's signed-ranks test, when they were measured immediately (around 15 min) after the one-time aromatherapy massage treatment, compared to the time point just before treatment (Fig. 1A). Interestingly, one-time intervention-induced EEG alterations were relatively weakened in the last eighth treatment compared to that of the first treatment (Fig. 1B). However, there were no substantial differences in the basal EEG patterns before the one-time aromatherapy massage between the first and the last eighth session (Fig. 1B). In addition, there were no significant differences in basal EEG pattern after 4 weeks based on ANCOVA analysis, suggesting that aromatherapy massage does not have long-term effects on such EEG pattern (Table 1). EEG changes were shown

**Table 1** The comparison of group differences after 4-week aromatherapy massage program.

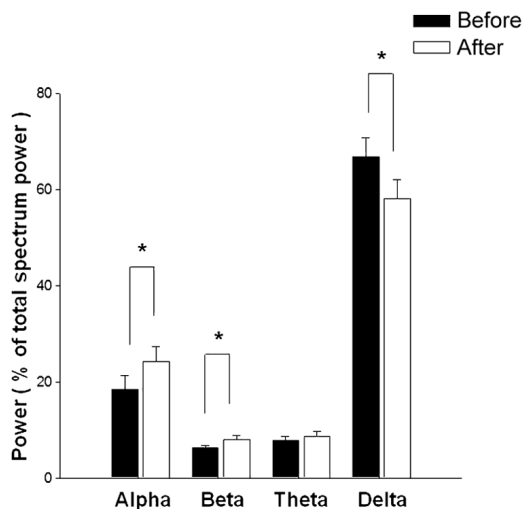
	Control (n=12)	Therapy (n=13)	p
<b>STAI-KYZ</b>			
Pre	88.5 ± 13.4	98.2 ± 23.0	0.01*
Post	85.3 ± 15.7	82.00 ± 22.3	
<b>BDI</b>			
Pre	8.6 ± 7.4	10.8 ± 9.9	0.04*
Post	8.5 ± 7.4	6.5 ± 7.5	
<b>PWI-SF</b>			
Pre	20.8 ± 10.0	24.3 ± 11.2	0.049*
Post	19.4 ± 10.0	17.6 ± 12.2	
<b>Alpha power (%)</b>			
Pre	17.6 ± 15.8	18.4 ± 10.6	0.06
Post	26.4 ± 14.8	16.3 ± 12.5	
<b>Plasma BDNF (pg/μl)</b>			
Pre	2336.9 ± 932.8	1885.2 ± 941.0	0.70
Post	2513.9 ± 1058.4	2630.2 ± 667.0	
<b>Plasma cortisol (μg/dl)</b>			
Pre	4.8 ± 0.2	4.6 ± 0.36	0.58
Post	4.6 ± 0.3	4.7 ± 0.5	

ANCOVA was used to analyze group differences between changes in the control and treatment groups after 4-week program. Values are presented as mean ± standard deviation (SD).

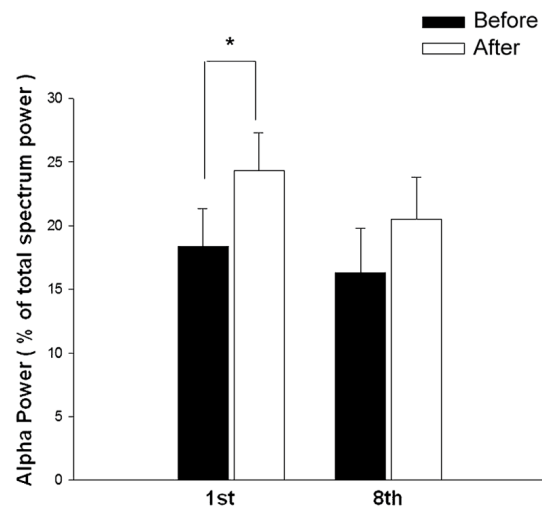
\* Significant difference ( $p < 0.05$ ) in STAI-KYZ, BDI, PWI-SF.

only when immediately tested after aromatherapy massage treatment (Fig. 1). Taken together, these data might be interpreted such that aromatherapy massage has a short-term potential in modulating the EEG pattern.

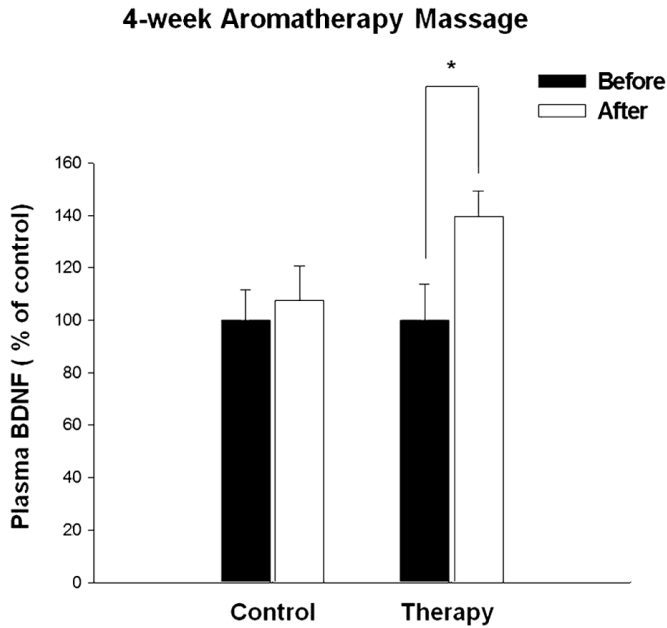
**A** One-time Aromatherapy Massage



**B** One-time Aromatherapy Massage



**Fig. 1** Short-term effects of one-time aromatherapy massage on EEG power spectrum pattern. (A) Effects of one-time aromatherapy massage treatment on EEG pattern were investigated using power spectrum analysis. The relative power of each of the four spectral bands including alpha (8–13 Hz), beta (13–25 Hz), theta (4–7 Hz) and delta (0.5–4 Hz) was expressed as mean ± SD percentage (%) of the total spectrum power (see Methods section), (B) The relative alpha power values were determined before and after one-time aromatherapy massage in the first and the last eighth intervention. \* $p < 0.05$ , when pre-post differences in the therapy group were compared using Wilcoxon's signed-ranks test.



**Fig. 2** Effects of 4-week aromatherapy massage program on plasma BDNF levels. The plasma BDNF levels were measured using EIA analyses before and after 4-week aromatherapy massage program in both the therapy and control groups and statistically analyzed. \* $p < 0.05$ , when pre-post differences in the therapy group were compared using Wilcoxon's signed-ranks test.

### Effects of aromatherapy massage on plasma BDNF levels

The basal plasma BDNF levels were measured using EIA analyses before and after the 4-week aromatherapy massage program in both the therapy and control

groups. Plasma BDNF levels were significantly ( $p = 0.03$ ) increased from 1625.0 (1241.7–2552.8)  $\text{pg}/\mu\text{l}$  to 2761.7 (2341.7–3187.2)  $\text{pg}/\mu\text{l}$  after a 4-week aromatherapy massage in the treatment group, compared to values before treatment, based on Wilcoxon's signed-ranks test (Fig. 2).

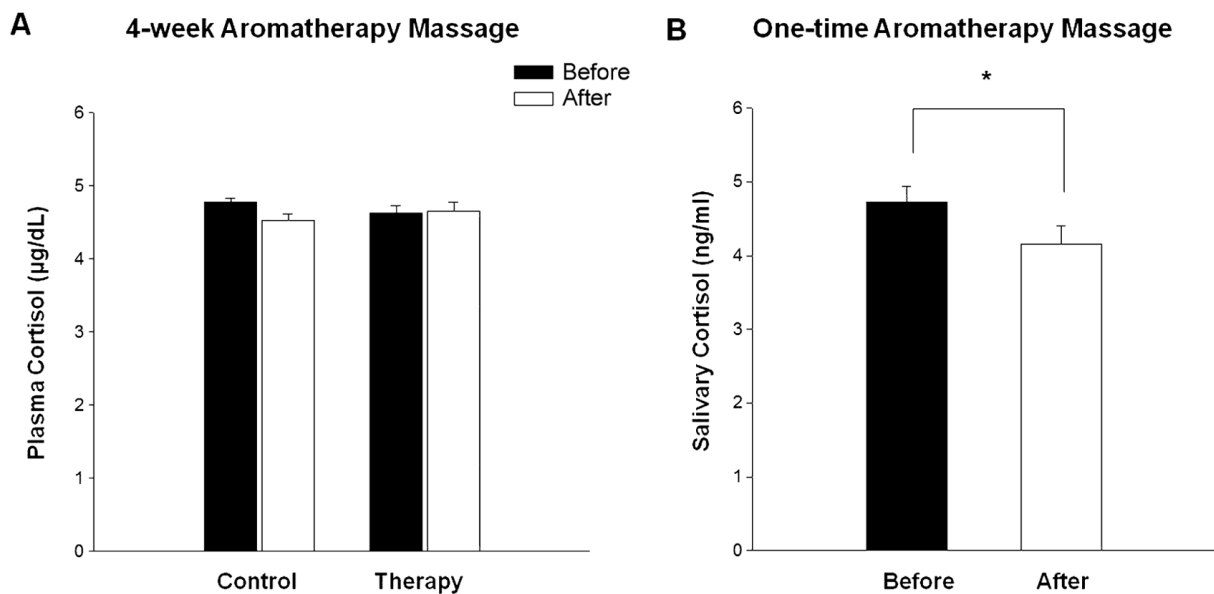
### Effects of aromatherapy massage on salivary cortisol levels

Salivary cortisol levels were significantly ( $p = 0.01$ ) decreased from 4.7 (4.3–5.3)  $\text{ng}/\text{ml}$  to 4.4 (3.5–4.8)  $\text{ng}/\text{ml}$  based on based on Wilcoxon's signed-ranks test, when they were measured at approximately 15 min after the one-time aromatherapy massage treatment, compared to the time point just before treatment (Fig. 3B). On the other hand, basal cortisol levels in plasma were not substantially changed after the 4-week aromatherapy massage program in both the control and therapy groups (Fig. 3A and Table 1). There were no significant differences in basal cortisol after 4 weeks based on ANCOVA analysis, suggesting that aromatherapy massage does not have long-term effects on cortisol levels. These data might be interpreted such that aromatherapy massage has a short-term potential in reducing cortisol levels, similar to those effects on EEG pattern (Fig. 1).

### Discussion

We demonstrated here that aromatherapy massage significantly exerted beneficial effects on the EEG spectral pattern, salivary cortisol and plasma BDNF levels as well as all of psychological assessment scores in STAI, BDI and PWI-SF inventories.

Psychological outcome measures such as STAI and BDI following aromatherapy inhalation have been published in



**Fig. 3** Short-term effects of one-time aromatherapy massage on cortisol levels. (A) Basal cortisol levels in plasma were measured using EIA analyses before and after 4-week aromatherapy massage program in both the therapy and control groups. (B) To examine effects of the one-time aromatherapy massage treatment on cortisol levels, salivary cortisol levels in the therapy group were determined using EIA analyses. Salivary samples were taken at the time points just before and after the one-time aromatherapy massage treatment. \*\*\* $p < 0.001$ , when pre-post differences in the treatment group were compared using Wilcoxon's signed-ranks test.



several studies. However, very few studies have been published following aromatherapy massage in cancer patients and palliative care.<sup>2,3</sup> Moreover, the studies regarding biological outcome measures following aromatherapy massage are very limited. Immunological effects have ever been published with STAI on healthy subjects following aromatherapy massage.<sup>4</sup> It was reported that massage intervention affected the maturation of brain electrical activity and increase of blood IGF in human infants and in rat pups.<sup>5</sup> However, this study is the first as far as we know that has measured the neurobiological effects of aromatherapy massage using EEG, salivary cortisol and plasma BDNF in humans.

The most interesting finding is that up-regulation of plasma BDNF levels as well as significant improvement of psychological outcome measures were induced by 4-week aromatherapy massage treatment. However, it should be mentioned that there were discrepancies in the statistical results on the BDNF analysis. There was no significant alteration in plasma BDNF levels after 4-week program based on ANCOVA putting emphasis on group changes (Table 1). Based on Wilcoxon's signed-ranks test (Fig. 2) to analyze the pre- and post-differences within treatment group, basal BDNF plasma levels were significantly increased after 4-week aromatherapy massage program.

BDNF, the most widely distributed and most abundant member of the neurotrophin family, is known to promote neuronal differentiation, survival, neurogenesis and synaptic plasticity. Recently, circulating BDNF levels has been paid attention to as a possible peripheral marker of depression. The plasma BDNF levels are decreased in untreated patients with major depressive disorders or neurodegenerative diseases in several clinical studies<sup>16</sup> and antidepressants increased serum BDNF levels after 6 months treatment.<sup>17</sup> On the other hand, stress and cortisol, a stress hormone on hypothalamus–pituitary–adrenal (HPA) axis, were reported to inhibit BDNF expression in the rat hippocampus.<sup>18</sup> Therefore, it might be postulated that eight times of aromatherapy massage interventions up-regulated plasma BDNF levels via negative regulation of cortisol at each session.

The neurological effect of aromatherapy massage on the EEG pattern has not ever been defined. Very few studies were done on EEG effect of inhalation aromatherapy and traditional Thai massage. Briefly, inhalation of rosemary essential oil decreased frontal alpha and beta power in EEG.<sup>19</sup> The traditional Thai massage induced an EEG change such as an increase in delta and a decrease in alpha and beta activities.<sup>20</sup> We demonstrated here that the alpha brain wave activities were enhanced and delta activities were significantly reduced after the one-time aromatherapy massage treatment. These EEG spectral patterns are similar to the typical alteration of brain rhythms shown in meditation and neurofeedback training.<sup>7</sup>

Taken together, we demonstrate here that aromatherapy massage might exert a beneficial effect on stress management and prevent stress-related psychiatric problems via body–brain interaction. However, there is a limitation in our study that each effect of massage treatment and aromatherapy was not to be distinguished in this experimental design. At least four groups should be necessary to distinguish each effect of massage therapy and aromatherapy in further studies: (1) massage alone (massages with inert

carrier oil), (2) aromatherapy and massage together (massages with essential oil), (3) inhalation aromatherapy alone (without massage), and (4) control (neither massage nor aromatherapy). It should be acknowledged that there is lack of equivalent placebo-control group to estimate an expectation effect against aromatherapy massage group in this study. In addition, because the small size of the control and therapy group could be another limitation, these issues should be perused with more participants in these above groups in the future study.

## Conclusions

Our results suggest that aromatherapy massage could exert a significant influence on multiple neurobiological indices, such as EEG power spectrum pattern, salivary cortisol and plasma BDNF as well as quantitative psychological assessments.

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