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Differences in Neurotransmitters Level as Biomarker on Sleep Effects in Dementia Patients with Insomnia after Essential Oils Treatment

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Abstract

This study aimed to examine the effects of several essential oils on insomnia in dementia patients following transdermal treatment (aromatherapy). The mean change rates (%) of sleep biomarkers were compared between the single essential and jojoba (vehicle) oil massage groups in this study. The lavender (L) essential oil massage group demonstrated a significant decrease in the mean change rate (%) of 24-h urinary free cortisol, whereas the valerian (V) essential oil massage group demonstrated a significant increase in the mean change rate (%) of serum 5-hydroxytryptamine. In addition, a significant increase in the mean change rate (%) of 24-h urinary norepinephrine was observed in the chamomile (C) essential oil massage group only. Based on these results, valerian, lavender, and chamomile oils were mixed in different ratios to produce blending oils A (L:C:V=2:2:1), B (L:C:V=3:1:1) and C (L:C:V=1:3:1). The highest level of serum 5-hydroxytryptamine was observed after administering blending oil A. These results suggest that blending oil A might possess therapeutic effects against insomnia. Overall, it is hypothesized that the optimally blended essential oil will produce synergic effects when combined with hypnotic drugs.

Key Words: Biomarkers, 5-Hydroxytryptamine, Free cortisol, Urine norepinephrine, Dementia patients, Insomnia

INTRODUCTION

Insomnia, a condition that affects 6-20% of the population in different countries, refers to the disturbance of sleep onset, maintenance, or quality (Ohayon, 2002). Unlike primary insomnia, secondary insomnia is a sleep disorder caused by underlying medical, neurological, or psychiatric conditions such as neurodegenerative diseases, including depression, Parkinson's disease (PD), Alzheimer's disease (AD), or dementia. The behavioral disturbances in dementia patients include insomnia, general tiredness, lethargy, irritability, and concentration or memory problems (Lack and Wright, 2015). The two most common forms of dementia are AD and vascular dementia, characterized by a general loss of neurotransmitters. Acetylcholine and many other neurotransmitters, including serotonin, norepinephrine (NE), dopamine, corticotrophin-releasing factor, and gamma-aminobutyric acid (GABA; in some studies), precipitously decline. It has been generally assumed that neuronal loss leads to neurotransmitter loss. Cholinesterase inhibitors (donepezil, galantamine, and rivastigmine), anticonvulsants and mood stabilizers (carbamazepine and divalproex), antidepressants (citalopram, fluoxetine, and trazodone), anxiolytics (buspirone and lorazepam), atypical antipsychotics (clozapine, olanzapine, quetiapine, and risperidone), typical antipsychotics (haloperidol), GABA agonists (benzodiazepines), and neuropeptides (corticotrophin-releasing factor) have been indicated in managing behavioral disorders to slow down or reverse the progression of dementia (Gabelle and Dauvilliers, 2010).

The prevalence of dementia has been approximately 25% higher since 2010 and doubles yearly (Gabelle and Dauvilliers, 2010). It was reported that approximately 25-35% of AD patients suffer from sleep disorders (Wang and Holtzman, 2020). Circadian disruption, melatonin dysregulation, and accumulation and aggregation of amyloid-beta (A-β) have a reciprocal relationship with insomnia in AD patients. (Bedrosian and Nelson, 2012). An increase in serum cortisol and NE was observed in AD patients (Bemelmans *et al*., 2007). Cerebrospinal fluid (CSF) NE also increases in AD patients due to decreased clearance and impaired reuptake of NE (Chal-

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bot *et al*., 2011). The loss of locus coeruleus (LC) neurons in AD causes a compensatory increase in NE activity (Chen *et al*., 2022), contributing to the arousal state, agitation, and anxiety (Elrod *et al*., 1997). Epinephrine is closely associated with sleep, wakening, and behavior. Similar to dorsal raphe nucleus (DRN) and substantia nigra pars compacta (SNc), the activity of serotonin neurons in non-rapid eye movement (NREM) is dependent on sleeping patterns, thereby increasing gradually if its active status during sleeping is slow and regular, or reaches the stage of wakening (Kumar *et al*., 2007; Monti and Jantos, 2008). In a study by Jones (2005), the cortisol level in the 24-h urinary excretion of a sleep-deprived person was increased (Riemann *et al*., 2002, 2020). Furthermore, a positive correlation was observed between 24-h urinary cortisol level and total wake time in patients with mild insomnia (Humer *et al*., 2020).

For the objective evaluation of the effects of essential oils (EOs) on sleep, 24-h urinary free cortisol, NE, and serum serotonin levels were used as sleep biomarkers in the present study. Hypnotic drugs such as benzodiazepine are used to manage insomnia. However, prolonged use of these drugs may lead to drug dependence, tolerance, bad sleep, rebound insomnia, deterioration in recognition function, and reduced effect (Shinomiya *et al*., 2005; McCall and McCall, 2012). However, alternative remedies are being employed to overcome these challenges. In aromatherapy, kava-kava, valerian, chamomile, ylang-ylang, melissa, hops, and lavender oils are being used to improve sleep (Wheatley, 2005). These oils have mild sleep-inducing effects without negative changes and a low rate of side effects. Hypnotic drugs like benzodiazepines are used as a short-term therapy, whereas EOs can be used for an extended period. Unlike zolpidem, α-pinene and limonene enhances the sleep quantity, decreases sleep latency, and increases NREM time. Distinct components from the monoterpenes group includes α-pinene and β-pinene. Those are present in the essential oil of various plants. Previously pinene has shown various pharmacological actions, few of them includes anxiolytic, neuroprotective and anticonvulsant effects (Salehi *et al*., 2019). α-Pinene is the major constituent that influences sleep (Yang *et al*., 2003, 2016). Chamomile roman oil demonstrated acute dermal toxicity when LD_{50} exceeded 5 g/kg in rabbits (Franke and Schilcher, 2005). Furthermore, linalool, one of the components of lavender oil, is known to possess sleep, anxiolytic, and anticonvulsant effects without regulating $GABA_A$ receptors; however, its exact mechanism has not yet been established (Brum *et al*., 2001). Due to its toxicity, lavender oil can be harmful when excessively used in pregnant women or children (Kim *et al*., 2011). It is known to cause dermatitis and allergic reaction in some populations. The lethal dose (LD_{50}) is 5 g/kg (dermal) in rabbits (Wheatley, 2005). In addition, limonene also affects barbital sleep (Yang *et al*., 2003, 2016). Thujone, a constituent of valerian oil, causes absinthism at the concentration of 260 mg/L; however, the concentration of thujone in valerian oil used in this study was low (0.00472 mg/L) (Lachenmeier *et al*., 2006). Jojoba oil mechanically extracted from *Simmondsia chinensis* by cold-press method. The constituents were reported at relatively high concentrations of 417 ppm, with gamma-tocopherol (79.2%), alpha, beta and delta-tocopherol. Furthermore, many practitioners recommend, jojoa oil, using a carrier to dilute the essential oils. It also helps with absorption and minimizes evaporation of the essential oils (El-Mallah and El-Shami, 2009). In some animal experiments, these oils were reported to have sleep effects; however, their pharmacological mechanism has not yet been fully understood, and the quality of sleep induced by EOs is yet to be evaluated using sleep biomarkers. Therefore, this study aimed to evaluate the effects of EOs on sleep quality and changes in neurotransmitters level, which are the sleep disorder biomarkers, in dementia patients with insomnia.

MATERIALS AND METHODS

Materials

The experimental materials used in this study include valerian, lavender, and chamomile roman oils, which have sleep effects. These EOs are the natural medicinal ingredients used for complementary medicine and massage products to improve sleep. These oils were purchased and analyzed from Absolute Aroma Co. (Hampshire, UK).

GS-MS analysis of essential oils

The constituents of EO extraction were analyzed by gas chromatography mass spectrometry (GC-MS) consisting of Agilent HP-6890 (Analytical Instrument Management, Littleton, CO, USA) gas chromatography coupled with Agilent HP-5973 mass selective detector equipped with a HP-5MS fused silica column (30 m×0.25 mm i.d., 0.25 μm film thickness). The GS conditions were used for the previously described analytical conditions (Jalali-Heravi *et al*., 2011). Each EO was diluted in jojoba oil (vehicle oil) to 3%. Jojoba and lavender oils are natural oils extracted from *Simmondsia chinensis* and *Lavandula angustifolia*, respectively. The main components of lavender oil are linalool (34.5%) and linalyl acetate (48.2%). It also includes a small quantity of α-pinene, 1,8-cineole, camphor, and others (Fig. 1A) (Elisabetsky *et al*., 1995; Cline *et al*., 2008). Valerian oil was extracted from the root of *Valeriana officinalis*. The main constituents of *Valeriana officinalis* are bornyl acetate (42.7%), camphene (29.6%), α-pinene (7.90%), β-pinene (5.05%), isoborneol (3.94%), isovalerate (4.22%) and carbonyl acetate (2.42%) (Fig. 1B). Chamomile roman oil was extracted from the flower of *Chamaemelum nobile L*. Its main constituents are 3-methylpentyl angelate (25.9%), 3-methylpentyl isobutyrate (17.1%), 2-methyl-2-propenyl angelate (13.1%), *trans*-pinocaneol (8.97%), and α-pinene (4.05%) (Fig. 1C). To minimize the valeric smell of valerian oil and improve sleep effects, the three different EOs were mixed in various ratios and diluted in jojoba oil to 3% (A=valerian: lavender: chamomile=1:2:2; B=valerian: lavender: chamomile=1:3:1; C=valerian: lavender: chamomile=1:1:3).

Study population

The study participants were 13 nursing home-dwelling dementia patients with a sleep disorder. These patients were diagnosed by neuropsychiatrists and were using urine bags or portable urinals. They were 71-94 years old (mean age: 84 years), with a male-to-female ratio of 6:7 (Table 1). This study was approved by the Sookmyung Women's University institutional review board (IRB) (Serial No. SM-IRB-12-0314-001). Informed consent was obtained from the legal representatives of all participants. This study was conducted in accordance with the declaration of Helsinki (World Medical Association).

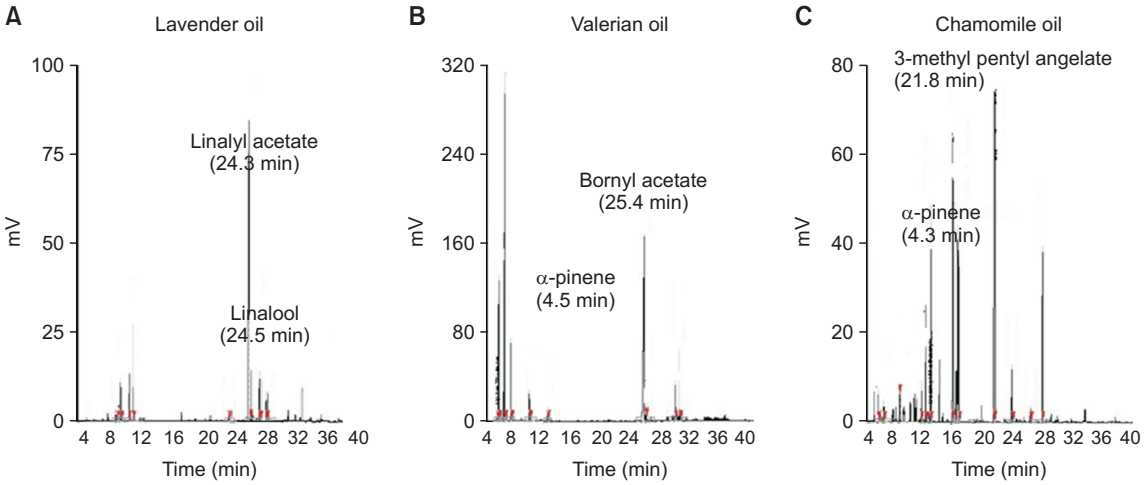

Fig. 1. GC-MS chromatograms of *Lavandula angustifolia*, *Valeriana officinalis* and Chamomile roman oils. (A) Lavender oil: principal constituents are linalool (24.3 min) and linalyl acetate (24.5 min). The constituents that increase sleep quality are α-pinene (4.4 min), limonene (8.8 min), and 1.8-cineole (9.22 min). (B) Valerian oil: main constituent is bornyl acetate (25.4 min). The constituents that increase sleep quality are α-pinene (4.5 min) and limonene (8.9 min). (C) Chamomile oil: main constituent is 3-methylpentyl angelate (21.8 min). The constituents that increase sleep quality is α-pinene (4.3 min).

Table 1. The comparison of sleep state pre and post massage of 3% essential oils in dementia patients which were measured for two weeks

Parameters		J	V	L	C
I	Pre	17 ± 1	15 ± 1	18 ± 1	19 ± 1
	Post	16 ± 1	10 ± 1**	15 ± 1	16 ± 1
	Change rate (%)	10 ± 2	73 ± 2**	86 ± 3	90 ± 1
N	Pre	11 ± 1	9 ± 1	8 ± 1	8 ± 1
	Post	10 ± 1	5 ± 1***	7 ± 1	6 ± 1
	Change rate (%)	86 ± 1	53 ± 1***	76 ± 3	80 ± 3
W	Pre	11 ± 1	11 ± 1	10 ± 1	10 ± 1
	Post	12 ± 1	7 ± 1***	9 ± 1	7 ± 1
	Change rate (%)	109 ± 2	60 ± 1***	85 ± 3*	85 ± 3

Each value represents the mean ± SEM (n=13). *p<0.05, **p<0.01, ***p<0.001, significantly different to pre and post. J, Jojoba; V, Valerian; L, Lavender; C, Chamomile; Insomnia severity index (I), day number of nap (N), day number of wake (W).

Sleep disturbance

Nurses assessed the sleep disturbance of all participants using daily sleep logs and insomnia severity index (ISI) scores for 2 weeks before and after aromatherapy. ISI (developed by Morin) scores were used to evaluate the severity of sleep disturbance. Cronbach's alpha was 0.89. Based on ISI scores, the participants were divided into four groups (a) normal insomnia (score: 0-7), (b) mild insomnia (score: 8-14), (c) serious insomnia (score: 15-21), and (d) severe insomnia (score: 22-28). Nurses recorded the frequency of awakenings every night, the presence of excessive daytime sleepiness daily, and total sleep hours.

The neuropsychiatric inventory–questionnaire (NPI-Q)

Neuropsychiatric inventory–questionnaire (NPI-Q) was developed and cross-validated using the standard NPI to provide a brief assessment of neuropsychiatric symptomatology in routine clinical practice settings. The NPI-Q is a self-administered questionnaire about the patients completed by the caregivers. Each of the 12 NPI-Q domains contains a survey question on the cardinal symptoms of that domain. The initial response to each domain question is "Yes" (present) or "No" (absent). If the response to the domain question is "No," the informant proceeds to the next question. If "Yes", the informant then rates the severity of the symptoms present within the last month on a 3-point scale and the associated impact of the symptom's manifestations on them (that is, caregiver distress) using a 5-point scale. The NPI-Q provides ratings on symptom severity and distress for each symptom reported. The total severity and distress scores are a summation of the individual domain scores. The NPI-Q was developed by Daniel Kaufer, MD, with permission.

The geriatric depression scale: short form (GDS: SF)

The geriatric depression scale: short form (GDS: SF) was developed in 1986 to screen for depression in older adults. It has been used in the community, acute, and long-term care settings. The GDS: SF consists of 15 questions requiring "yes" or "no" answers that can be completed quickly. GDS: SF of 0-4 is negligible, 5-8 suggests mild depression, 9-11 suggests

moderate depression, while 12-15 suggests severe depression.

National sleep foundation sleep diary

The national sleep foundation is dedicated to improving health and well-being through sleep education and advocacy. It is renowned for its annual Sleep in America® poll. The national sleep foundation sleep diary helps track a person's sleep by allowing the individual to see habits and trends that aid sleep or that can be improved.

Neuroendocrine biomarkers measurement of physiologic arousal

Neuroendocrine biomarkers are diagnostic criteria in insomnia patients. The hypothalamic-pituitary-adrenal (HPA) axis may take a role of the stress response system, therefore the chronic activation of HPA result from insomnia. So researchers (Michael *et al*., 2005) had also measured neuroendocrine biomarkers including norepinephrine and free cortisol to examine as potential correlates of insomnia through urinary and plasma sample.

Urinary measurement

Free cortisol level and NE of 24-h urine were measured. A subsequent study of urinary cortisol and epinephrine found no significant difference between middle-aged good and poor sleepers. However, poor sleepers showed a trend toward higher urinary cortisol and NE. A study by Vgontzas *et al*. (1998) showed that 24-h urine cortisol, NE, 3,4-dihydroxyphenylacetic acid (DOPAC), 3,5-dihydroxyphenylglycine (DHPG), and growth hormone (GH) levels were positively associated with total wake time in 15 patients with insomnia.

Plasma measurement

A study examining the effects of NE on sleep regulation in cats reported that NE positively affects the arousal mechanism (Kumar *et al*., 2007). In another study, five patients with a high degree of sleep disturbance (sleep efficiency <70%) secreted higher amounts of cortisol than those with less sleep disturbance (Riemann *et al*., 2002). Low serotonin levels are believed to be associated with depression and insomnia. When the serotonin level is normal, sleep falls into place in patients with sleep disturbances (Riemann *et al*., 2020). Serotonin is significantly associated with sleep/wake regulation (Humer *et al*., 2020). The plasma level measurement was selected as a serotonin sleep-related biomarker (Al-Sharman *et al*., 2021).

Study design

A skin test, blood pressure check, and body temperature check were performed for all participants before aromatherapy. Simple time series and multiple component designs were used. The multiple component designs were: (a) baseline (b) jojoba treatment (c) break time (d) 3% valerian treatment (e) break time (f) 3% lavender (g) break time (h) 3% chamomile oil (i) break time (j) 3% mixed oil A (k) break time (l) 3% mixed oil B, (m) break time (n) 3% mixed oil C. The duration of each stage was two weeks. During the 2-week baseline period, nurses collected blood samples and 24-h urine. There was a break time between each 2-week aromatherapy course. EOs used for the aromatherapy were diluted in jojoba oil to 3%. The baseline was used as a control, and jojoba oil was used as a placebo.

Sample collection and treatments

Blood was collected at 9:00 a.m., and 24-h urine collection commenced at 9:00 a.m. Nurses massaged the participants' faces and hands for 1-2 min 1-2 h before their scheduled bedtime for 2 weeks. Furthermore, 24-h urine was collected before each 2-week aromatherapy course commenced and the next day upon completing the 2-week aromatherapy course. Serum cortisol, serotonin, NE, and free cortisol levels of 24-h urine pre- and post-interventions were analyzed. Then, 3 mL of blood was collected in a serum-separating tube (SST) and coagulated for 30 min. Next, 1.5 mL of serum was transferred to a serum separator and stored in a freezer. After 14 days, the samples were thawed. A minimum of 1 mL untreated urine was collected in a serum separator or a 1.5 mL tube and stored in a freezer to check the free cortisol level of 24-h urine. Next, 2% 6N-HCl was added to the samples, and a minimum of 5 mL of urine was collected in a serum separator or 1.5 mL tubes to check the NE level of 24-h urine. Then, they were stored in a freezer.

Analytical method of serotonin, free cortisol, and norepinephrine

Sample analysis methods include high-performance liquid chromatography (HPLC; Clin Rep® Complete kit, Shimadzu/Hitachi, Tokyo, Japan) used for the previously described analytical conditions (Benmansour *et al*., 1999). Fluoroimmunoassay was used to analyze 24-h urine free-cortisol and NE, and Green Cross Research Institute performed the analysis.

Statistical analysis

All the result data were presented as mean ± standard deviation. Paired t-test was used to analyze the data. Statistical significance was set at $p<0.05$. Statistical analysis was performed using excel.

RESULTS

The neurotransmitters as sleep biomarkers and the sleeping patterns in dementia patients before treatments (dementia patient's baseline)

The average serum serotonin level before aromatherapy was 107 ± 4 ng/mL (standard value: <200 ng/mL). The mean 24-h urinary free-cortisol level was 32.0 ± 2.2 μg/day (standard value: 20-90 μg/day). The mean 24-h urinary NE level was 20.6 ± 0.9 μg/day (standard value: 15-80 μg/day). The mean number of days taking a nap within 2 weeks was 11 ± 1. The arousal frequency was 10 ± 1. The mean ISI score was 17 ± 1. There was a 14-day break interval between the 2-week aromatherapy courses.

The changes in biomarkers and sleeping patterns before and after treatments

There was no significant difference in the serum serotonin levels (Fig. 2) after single oil therapy. There was a significant decrease in the free cortisol level of 24-h urine after aromatherapy using lavender oil (Fig. 3). There was a significant increase in the NE levels of 24-h urine after aromatherapy using chamomile oil (Fig. 4). A significant decrease in the ISI score, the frequency of night awakenings, and the number of excessive daytime sleepiness (10 ± 1, 4.5 ± 0.1, and 6.8 ± 0.2, respectively) was observed after aromatherapy using va-

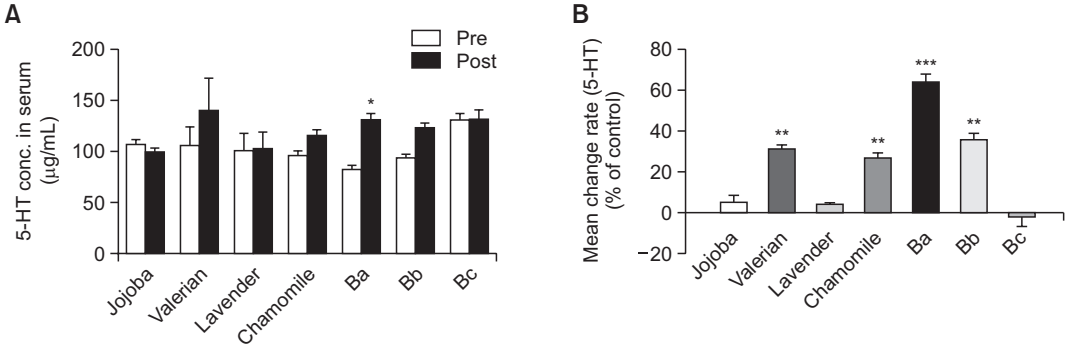

Fig. 2. 5-HT concentration and mean change rate in serum. (A) The change in serum concentration of 5-HT pre- and post-massage using essential oils in dementia patients (n=13). Ba: blending oil A (V:L:C=1:2:2), Bb: blending oil B (V:L:C=1:3:1), Bc: blending oil C (V:L:C=1:1:3). **p*<0.05, significant difference between pre and post. (B) Mean change rate compared with pre-concentration of neurotransmitter 5-HT in serum. ***p*<0.01, ****p*<0.001 significantly different from pre concentration of 5-HT serum.

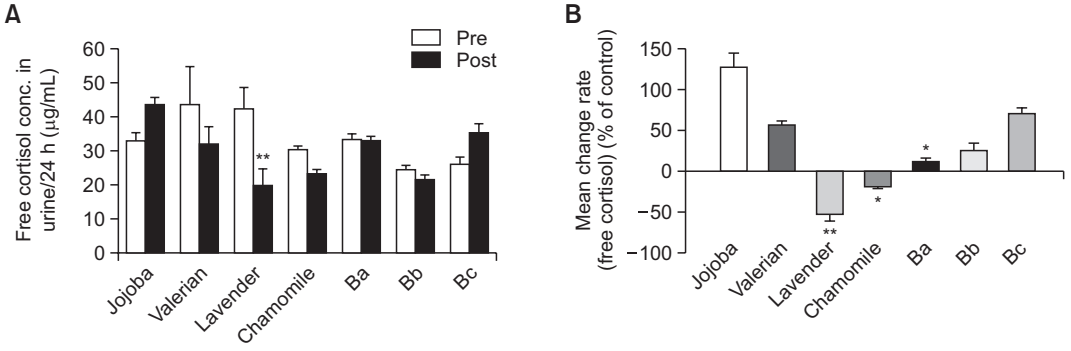

Fig. 3. Cortisol concentration and mean change rate in urine. (A) The change in 24-h urine free cortisol concentration pre- and post-massage using essential oil in dementia patients. Ba: blending oil A (V:L:C=1:2:2), Bb: blending oil B (V:L:C=1:3:1), Bc: blending oil C (V:L:C=1:1:3). ***p*<0.01, significant difference between pre and post. (B) Mean change rate compared with pre-concentration of neurotransmitter free cortisol in urine. **p*<0.05, ***p*<0.01 significantly different from pre concentration of free cortisol in urine.

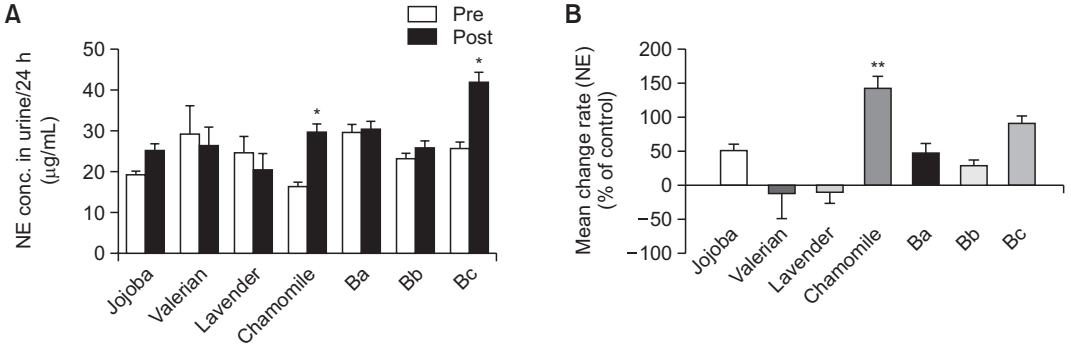

Fig. 4. NE concentration and mean change rate in urine. (A) The change in 24-h urine NE concentration pre- and post-massage using essential oil in dementia patients. Ba: blending oil A (V:L:C=1:2:2), Bb: blending oil B (V:L:C=1:3:1), Bc: blending oil C (V:L:C=1:1:3). **p*<0.05, significant difference between pre and post. (B) Mean change rate compared with pre-concentration of neurotransmitter NE in urine. ***p*<0.01 significantly different from pre concentration of NE in urine.

lerian oil, indicating the improvement in sleep quality. Only the number of days of taking a nap significantly decreased after aromatherapy using lavender oil (Table 1).

Pre-to-post changes in biomarkers after aromatherapy using single oil

The biomarkers of the control and experimental groups pre- and post-interventions were compared. After single aromatherapy using valerian and chamomile oils, there was a significant increase in serum serotonin level (Fig. 2B). After single

Table 2. The comparison of sleep state pre and post massage of 3% blending oils in dementia patients which were measured for two weeks

Parameters		Ba	Bb	Bc
I	Pre	15 ± 1	14 ± 1	14 ± 1
	Post	10 ± 1*	11 ± 1*	13 ± 1
	Change rate (%)	69 ± 2**	83 ± 1*	89 ± 12
N	Pre	9 ± 1	8 ± 1	8 ± 1
	Post	4 ± 1***	5 ± 1**	6 ± 1
	Change rate (%)	45 ± 1***	48 ± 1***	87 ± 2
W	Pre	12 ± 1	12 ± 1	11 ± 1
	Post	5 ± 1***	6 ± 1***	10 ± 1
	Change rate (%)	46 ± 1***	46 ± 1***	94 ± 2*

Each value represents the mean ± SEM (n=13). *p<0.05, **p<0.01, ***p<0.001, significantly different to pre and post. Paired t-test were performed. A: (L:C:V=2:2:1), B: (L:C:V=3:1:1), C: (L:C:V=1:3:1), Insomnia severity index (I), day number of nap (N), day number of wake (W).

aromatherapy using lavender and chamomile oils, there was a significant decrease in the free cortisol level of 24-h urine (Fig. 3B). Only Chamomile oil induced a significant increase in NE of 24-h urine (Fig.4B).

Pre-to-post changes in biomarkers after aromatherapy using mixed oils

The three different oils were blended to reduce the unpleasant smell of valerian oil, which had the most significant sleep effects. After blending oil A (Ba; L:C:V=2:2:1) treatments, the serum serotonin level was significantly increased (Fig. 2A); however, there was no change in free cortisol levels of 24-h urine (Fig. 3A). After blending oil C (Bc; L:C:V=1:3:1) treatment, there was a significant increase in NE levels of 24-h urine (Fig. 4A).

Pre-to-post change rates of biomarkers using mixed oils

After aromatherapy using blending oil B (Bb; L:C: V=3:1:1), which contained 0.6% valerian oil, the highest level of serotonin was observed with the pre-to-post change rate of 64 ± 1% (Fig. 2B), and the increase was double the effect of 3% valerian oil. In contrast, the free cortisol of 24-h urine was significantly reduced (Fig. 3B). Except for Bc, treatment with Bb also significantly increased the serotonin level (Fig. 2B).

Changes in insomnia level and sleeping pattern using mixed oils

After aromatherapy using Ba and Bb, there was a significant decrease in the ISI score, the frequency of night awakenings, and the number of days of taking a nap (Table 2). Comparing the pre-to-post changes induced by the mixed oil treatment with those of the placebo, it was concluded that aromatherapy using Ba and Bb has good sleep effects (Table 2).

Comparison of NPI-Q and GDS: SF after essential oils massage

NPQ-1 and GDS: SF evaluations revealed a significant decrease in abnormal behaviors such as anxiety, depression, alertness, and wandering (Table 3). This is due to increased plasma serotonin levels and reduced urine cortisol concentrations (Fig. 2B, 3B).

Table 3. The comparison of MMSE-K and NPI-Q, SGDS after essential oils massage

	MMSE-K	NPI-Q	SGDS
Pre	12 ± 4	8 ± 6	9 ± 2
Post	11 ± 4	2 ± 2*	2 ± 1**

Each value represents the mean ± SEM (n=13). *p<0.01, **p<0.001, significantly different to pre and post. Paired t-test were performed. MMSE-k: mini-mental status examination-korea. NPI-Q: neuropsychiatric inventory questionnaire, SGDS: Short form of geriatric depression scale (Korean version of the short form of Geriatric Depression Scale: SGDS-K).

DISCUSSION

Secondary insomnia is associated with the disrupted function of neurobiological basis. Peripheral blood platelets are considered a model of the central 5-hydroxytryptamine (5-HT) neurons. Both plasma and central 5-HT have similar dynamics of 5-HT. Platelet (Sviglin *et al*., 2011) melatonin was produced from 5-HT daily at sunset and throughout the night. Melatonin level is highest at night. Melatonin, often referred to as the sleep hormone, is a central part of the body's sleep-wake cycle. Its production increases with evening darkness, promoting healthy sleep and helping to orient our circadian rhythm. Low serotonin production is equivalent to Low melatonin production, and a low amount of melatonin results in poor sleep (Hardeland, 2012). L-Tryptophan is converted to 5-HT in serotonin, which is further converted to N-acetyl-5HT and then finally to melatonin. 5-HT is converted to melatonin by two enzymes, 5-HT N-acetylate and hydroxyindole-O-methyl transferase (Lee *et al*., 2014). 5-HT modulates the sleep/wake cycle. Levels of 5-HT are always low at night and high during the day. Furthermore, several studies have reported an interaction between insomnia and free cortisol in plasma and urine (Field *et al*., 2008).

The psychoactive volatiles from these EOs conform to five conditions: (a) molecular weight (MW) <500, (b) polar surface area <140A (Setzer, 2009), (c) octanol-water partition coefficient with log p<5, (d) no > five hydrogen bond donors, (e) no >10 hydrogen bond acceptors. EO components' MW is approximately 250 and lipophilic; therefore, volatile EO can cross the blood-brain barrier (BBB) (Agatonovic-Kustrin *et al*., 2020).

The present study evaluated the effects of valerian, lavender, and chamomile oils on insomnia using biological biomarkers, such as 5-HT, NE, and free cortisol, daily sleep logs, and ISI scores. After aromatherapy using lavender oil, 24-h urine-free-cortisol level was reduced. A previous study reported a significant decrease in cortisol levels in saliva and sleep induction after a bath with lavender oil in pregnant women and young infants (Field *et al*., 2008; Wang *et al*., 2022).

Furthermore, the inhalation of lavender oil was conducive to maintaining sleep-inducing properties by the increase in alpha-power in the electroencephalogram (EEG), which occurs during relaxation owing to repetitive injection in patients with stress-induced insomnia (Wheatley, 2005). A study by Lewith reported that sleep quality improved after 4 weeks of lavender oil inhalation in 10 people (Lewith *et al*., 2005). The present study showed that aromatherapy using chamomile oil reduced sleep quality by increasing the NE level of 24-h urine (143%),

causing wakening despite the 27% increase in serum serotonin level. This result is consistent with that of Shinomiya *et al*. (2005), reporting the minimal sedative effects of chamomile roman oil, which is being used in inhalers, massage therapy, and drinks, compared with the control and diazepam (Lewith *et al*., 2005; Wheatley, 2005).

Normal sleep EEG pattern is divided into REM and NREM. NREM is further divided into four stages: stages I, II, III, and IV. Lavender oil demonstrated lesser effects on the delta wave, which occurs in a deep sleep (slow wave sleep [SWS]): sleep stages III and IV). High doses of valerian oil, such as 1,500 mg, led to a power increase in delta and a decrease in alpha- and beta-power (Vonderheid-Guthl *et al*., 2000). Valreranon, the major component in valerian oil, caused sleeping, improved sleeping time, decreased anxiety, and increased pentobarbitone-induced sleep time in larger doses (such as 100 mg/kg). Valerian oils were reported to increase slow-wave sleep, decrease sleep stage I, increase sleep stage III, and decrease REM sleep. Changes in the beta-intensity exhibited hypnotic effects. Valerian oil extract decreased night awakening and improved sleep quality (Shinjyo *et al*., 2020).

Valerian oil has a synergic effect with pentobarbital sodium by inhibiting the activity of the autonomic nervous system and expanding SWS and REM stages (Wheatley, 2005). After aromatherapy using 3% valerian oil, there was a 31% increase in the serotonin level. Valerian oil increased the concentration of GABA and 5-HT at the synapse (Vonderheid-Guthl *et al*., 2000). Valerian oil also increased the serum levels of 5-HT and GABA and decreased the DA level in the hippocampus of rats (Wenfei *et al*., 2022). An improvement in sleep was characterized by increased serum 5-HT level and reduced 24-h urinary NE and free cortisol levels. Furthermore, the serum serotonin level was increased to approximately 64% with the level of 140 ng/mL (standard value: <200 ng/mL) after aromatherapy using the Ba, containing 0.6% valerian oil. This increase was double that of the effect of 3% valerian oil treatment. This result had a higher increase rate (14%) than a case of administering serotonin reuptake inhibitor, which is being used as a therapeutic drug and is at the normal level. In addition, there was no significant increase in the NE level, which has an arousal effect (Dumont *et al*., 2005). According to the International Aroma Association and the Cosmetics Stability Standard (FDA, MD, USA) for dermal toxicity, EOs should be used after diluting to 3%. Therefore, the Ba is the optimal ratio manifesting pharmacological effects by increasing serum 5-HT level and reducing 24 h urinary NE and free cortisol levels. The decrease in NPQ-1 and SGDS evaluation, as shown in Table 3, indicated abnormal behavior, such as anxiety, depression, alertness, and wandering, resulting from increased intra-plasma serotonin levels and reduced intra-urine NE and cortisol concentrations. Decreased anxiety, depression, alertness, and wandering alleviate insomnia. The combination of short-term sleeping pills and this blending oil reduces side effects such as tardive dyskinesia, anticholinergic reaction, Parkinsonian event, orthostatic hypotension, cardiac conduction, disturbance, reduced bone mineral density, sedation, and cognitive slowing (Keepers *et al*., 2020).

This study revealed valerian oil is associated with increased serum serotonin levels and good quality sleep. Valerian oil and the blending oils A also affect the duration of sleep time in patients because the frequency of night awakenings decrease. Dementia patients were able to sleep an average of 6-7 h a day in this study. However, it is not an ideal option due to its pungent smell and the toxic substance called thujone, which must be migrated into small amounts. Similar to drugs, the mixture of valerian, lavender and chamomile oils (Ba) in an appropriate ratio improved insomnia. Increased serum serotonin level and decreased 24-h urine cortisol level may indicate sleep quality. However, further studies needed to establish the relationship between the effects of EOs and the mechanisms of the nervous system and neurotransmitters. Improving sleep disturbance in patients with dementia may reduce caregiver burden and resolve dementia patients' suffering. In treating insomnia, the mixture of oils may be appropriate for long-term sleep hygiene and drugs of diverse dosage forms without medication side effects.

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