

Effects of Bergamot (*Citrus bergamia* (Risso) Wright & Arn.) Essential Oil Aromatherapy on Mood States, Parasympathetic Nervous System Activity, and Salivary Cortisol Levels in 41 Healthy Females

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Keywords

Bergamot essential oil (BEO) · *Citrus bergamia* (Risso) Wright & Arn. · Psycho-endocrinological study · Salivary cortisol · Sympathetic nervous system · Randomized crossover clinical trial

Summary

Background: Bergamot essential oil (BEO) is commonly used against psychological stress and anxiety in aromatherapy. The primary aim of the present study was to obtain first clinical evidence for these psychological and physiological effects. A secondary aim was to achieve some fundamental understanding of the relevant pharmacological processes. **Methods:** Endocrinological, physiological, and psychological effects of BEO vapor inhalation on 41 healthy females were tested using a random crossover study design. Volunteers were exposed to 3 experimental setups (rest (R), rest + water vapor (RW), rest + water vapor + bergamot essential oil (RWB)) for 15 min each. Immediately after each setup, saliva samples were collected and the volunteers rested for 10 min. Subsequently, they completed the Profile of Mood States, State-Trait Anxiety Inventory, and Fatigue Self-Check List. High-frequency (HF) heart rate values, an indicator for parasympathetic nervous system activity, were calculated from heart rate variability values measured both during the 15 min of the experiment and during the subsequent 10 min of rest. Salivary cortisol (CS) levels in the saliva samples were analyzed using ELISA. **Results:** CS of all 3 conditions R, RW, and RWB were found to be significantly distinct ($p = 0.003$). In the subsequent multiple comparison test, the CS value of RWB was significantly lower when compared to the R setup. When comparing the HF values of the RWB setup during the 10 min of rest after the experiment to those of RW, this parameter was significantly increased ($p = 0.026$) in the RWB setup for which scores for negative emotions and fatigue were also improved. **Conclusion:** These results demonstrate that BEO inhaled together with water vapor exerts psychological and physiological effects in a relatively short time.

Schlüsselwörter

Ätherisches Bergamotte-Öl · *Citrus bergamia* (Risso) Wright & Arn. · Psychoendokrino-logische Studie · Speichel-Cortisol · Sympathikus · Randomisierte Crossover-Studie

Zusammenfassung

Hintergrund: Ätherisches Bergamotte Öl wird im Rahmen der Aromatherapie gegen psychischen Stress und bei Angstzuständen eingesetzt. Das primäre Ziel der vorliegenden Studie war es, erste klinische Belege dieser Effekte zu generieren. Ein weiteres Ziel bestand darin, die pharmakologischen Wirkprinzipien der daran beteiligten Prozesse zu erfassen. **Material und Methoden:** Die endokrino-logischen, physiologischen und psychologischen Effekte der Dampfinhalation von ätherischem Bergamotte-Öl wurden in einer randomisierten Crossover-Studie an 41 gesunden Frauen getestet. Die Probandinnen wurden in 3 Versuchsgruppen aufgeteilt: (Ruhe (R), Ruhe + Wasserdampf (RW), Ruhe + Wasserdampf + ätherisches Bergamotte-Öl (RWB)). Jede Sequenz dauerte 15 min. Unmittelbar nach jedem Set wurden den Studienteilnehmerinnen Speichelproben entnommen und eine 10-minütige Ruhephase verordnet. Im Anschluss daran füllten die Testpersonen das Profile of Mood States, das State-Trait-Angstinventar und die Fatigue Self-Check-Liste aus. Die hohen Herzfrequenzwerte, die als Indikator für die Parasympathikus-Aktivität gelten, wurden anhand der Herzfrequenzvariabilitätswerte kalkuliert und während des 15-minütigen Experiments sowie der anschließenden 10-minütigen Ruhephase erhoben. Die Level des Speichel-Cortisols (CS) aus den Speichelproben wurden mittels ELISA analysiert. **Ergebnisse:** Die CS in allen 3 Bedingungen (R, RW und RWB) unterschieden sich signifikant voneinander ($p = 0,003$). Der CS-Wert bei RWB erwies sich im multiplen Vergleichstest mit R als signifikant niedriger. Der Vergleich der HF-Werte im RWB-Versuchsaufbau mit den RW-Werten während der 10-minütigen Ruhephase zeigte einen signifikanten Anstieg dieses Parameters unter der RWB-Kondition ($p = 0,026$). Dabei verbesserten sich auch die Scores für negative Stimmungen und Müdigkeit. **Schlussfolgerung:** Die Ergebnisse zeigen, dass die kombinierte Inhalation von ätherischem Bergamotte-Öl und Wasserdampf sowohl psychisch als auch physisch positive Effekte innerhalb einer relativ kurzen Zeitspanne hervorruft.

Introduction

Bergamot (*Citrus bergamia* (Risso) Wright & Arn.; syn. *Citrus aurantium* L. var. *bergamia* Loisel.), which belongs to the Citreae tribe in the Aurantioideae subfamily of the Rutaceae plant family, originates from the Mediterranean ecoregion, particularly from southern Italy and Greece. Its volatile oil, which is produced from the exocarp by means of cold pressing [1], is in high demand for a wide range of perfumes, cosmetics, and especially for aromatherapy [2, 3].

According to recent research in a combination of fields, such as phytochemical analysis, pharmacology, and psychology, a variety of volatile oils have specific neuropharmacological effects [4]. The individual volatile oil compounds are detected by the nervus olfactorius on the back of the nasal cavity, which carries more than 1,000 kinds of receptors and is directly connected via the intracranial olfactory bulb to the limbic system in the hypothalamus and thus to the autonomic nervous system. Accordingly, effects on the endocrine and immune systems have been demonstrated [5]. Furthermore, volatile oil components inhaled during aromatherapy can pass from the alveoli into the capillary blood vessels [5]. Even if rubbed onto the skin, they can enter through the subcutaneous tissue into the capillaries and pass the blood-brain barrier with the bloodstream, thus affecting the entire central nervous system [5]. Volatile oils were shown to be especially effective in the therapy of chronic pain [6, 7], depression [8, 9], cognitive disorders [10, 11], insomnia [12], and stress-related ailments [13]. They were further demonstrated to exert antibacterial, antifungal, antiviral, and cytotoxic effects [14].

Bergamot volatile oil is commonly regarded as an effective traditional pharmaceutical and medicinal product that was used for many centuries against a variety of conditions. Today, it is widely used in aromatherapy for reducing stress and anxiety [3]. According to most recent research, it counteracts anxiety behavior in rats via the stress response pathway, reducing the activity of the hypothalamus-pituitary-adrenocortical axis (HPA) [3]. Furthermore, most recent studies using the forced swim and open field tests have demonstrated the taxonomically identical TCM drug fructus aurantii (Chin.Ph.) to exert significant antidepressant effects via the monoaminergic mechanism in rats [15]. However, despite the common use of bergamot volatile oil water vapor, its psychological and endocrinological effects in humans have not been subject to experimental investigation up to now.

When a stressor reaches the cerebral cortex, adrenocorticotrophic hormone (ACTH) is released from the hypothalamus stimulating the secretion of cortisol from the adrenal cortex [16]. Based on this knowledge, we have already used salivary cortisol (CS) levels as an indicator for stress reduction in previous research projects, demonstrating that during emotional improvement after relaxation cortisol levels are also reduced [17, 18]. In a preliminary study related to the present project, these changes in the CS levels of individual test subjects under bergamot aroma therapy were shown to be closely linked to their individual personalities and lifestyles [19].

In the present randomized crossover study, 41 healthy young adult women were given bergamot volatile oil-saturated water vapor to inhale, while their CS level was measured repeatedly. Both heart rate variability as an indicator for the activity of the autonomic nervous system and emotional state were monitored.

Material and Methods

Study Participants

Originally, 42 healthy female graduate and undergraduate university students, all between 20 and 23 years old and with an average age of 21.3 ± 1.02 years, were recruited for the present human trial. This number was based more on financial than on statistical considerations as we tried to recruit as many participants for the study as our budget did allow. As one of the recruited volunteers did not show up on the day of the experiment, the study was carried out with the remaining 41 subjects. In order to prevent influences of age or gender on the data, age and gender of the test subjects had been equalized, and also their general living conditions as they are relatively similar among university students. The experimental protocols were approved by the only currently established Ethics Committee of the Kyoto Prefectural University of Medicine in full accordance with the Declaration of Helsinki in the most recent revised and amended version of 2013.

All test subjects were informed in detail about the purpose of the experiment and about the overall experimental setup. After receiving detailed information, such as a plan of the intended study similar to table 1, all recruited individuals manifested their intent to participate in the study by signing the respective test consent forms.

Each participant gave written informed consent and completed a medical health questionnaire prior to participation. Before the study, the volunteers had to fill out a questionnaire concerning sleep and wake-up time for the day of the investigation itself, general health, mental stress, energy, time of their most recent menstrual period, if they had eaten breakfast or not, and when they had eaten for the most recent time to make sure that all of them were in normal mental and physical state.

Study Design

The study was designed as a randomized crossover trial. In order to eliminate effects caused by the mere sequence in which the experiments were performed, the 42 study participants had originally been divided into 6 groups, consisting of 7 volunteers each, so that every 7-person group could perform 1 of the 6 possible permutations of the 3 setups – rest (R), rest + water vapor (RW), and rest + water vapor + bergamot oil (RWB). All study participants were assigned to 1 of these groups using a random number table generated in Microsoft Excel. The same applied for the sequence in which the individual groups took part in the experiment. The person who did not show up at the day of the experiment had originally been assigned to the RWB, R, RW permutation, which was therefore performed by only 6 volunteers, whereas each of the 5 other permutations was performed by groups consisting of 7 test subjects. The first group took part in the setups with R, RW, RWB; the next group in the sequence RWB, R, RW; the next in the sequence RW, RWB, R; and so forth, until all 6 groups had finished their respective permutation. Each participant took part in the study only once, completing all 3 setups in a row. The study was conducted with 1 group per day under exactly identical measuring conditions for all parameters.

The test persons were exposed to the 3 study setups R, RW, and RWB for 15 min each (table 1) followed by 2 min of rest for the collection of saliva samples, 10 min for continued recording of the heart rate variability, and 10 min for filling out the questionnaires. In order to limit interferences in the data that can be caused by physical exercise or nervousness, the test subjects took a rest of 15 min before the start. The experiments took 3 h from the first explanations to the volunteer until the disconnection of the heart rate recording device. On each day of the study, work began at exactly 1:00 pm and proceeded in the fashion

Table 1. Schematic depiction of the experiment procedure. The sequence in which the rest, rest + water vapor, and rest + water vapor + bergamot oil settings were performed was randomized (6 permutations) in order to exclude an influence of the experimental sequence on the study outcome. The distribution of the 41 volunteers between the 6 groups and the order in which the groups participated in the test were randomized using a random number sequence generated in Microsoft Excel.

Sequence	min
◇ rest	15
◇ rest (R)	15
★ saliva collection	2
◇ rest	10
⊙ questionnaire	10
◇ rest + water vapour (RW)	15
★ saliva collection	2
◇ rest	10
⊙ questionnaire	10
◇ rest + water vapour + bergamot oil (RWB)	15
★ saliva collection	2
◇ rest	10
⊙ questionnaire	10
◇ measurement of heart rate variability, ★ saliva collection, ⊙ completed questionnaire.	

described above. The test subjects were advised not to eat anything from 1 h before the start of the experiment. The experimental schedule was conducted as depicted in table 1.

During the experiment, the test subjects were required to sit on a chair, keep their hands on a table in front of them, and their body in a stable position. They were forbidden to talk, move, drink, eat, or do anything else that was not related to the experiment. Also when resting, the volunteers had to keep sitting on the chair. The applied diffusor (Tree of Life, Tokyo-Shibuya, Japan) diffuses water vapor into the air using ultrasound waves. When volatile oils are inserted into the system, they are dispersed in the air together with the water. During the experiment, the apparatus was placed on the table in front of the volunteers 30 cm away from the face. During the R setup, it was switched on with the tank being empty, in the RW setup the tank was filled with water (purified tap water using a common carbon adsorption filter) so that only water vapor was produced. In the RWB setup, bergamot essential oil (BEO) was applied by giving 400 µl of BEO into the 400 ml tank of the diffusor, dispersing it in the air together with the water. This dosage of BEO was determined on the basis of previous preliminary test results [19].

Measuring conditions for room temperature and overall humidity were kept constant at 22°C and 50% using a conventional air conditioning system and heating-type humidifier. Between the individual setups, all windows were opened and the room vented with an industrial fan (Yamazen, 45cm YKY-454) until all participating experimenters unanimously judged the bergamot smell to have completely disappeared. Thereafter, the setups without bergamot oil vapor were conducted.

In the present experiment, the physiological and psychological parameters described below were analyzed in order to detect potential differences between the 3 experimental setups.

Phytochemical Analysis of BEO

BEO (Lot: LF1481211) was produced from original Italian bergamot fruits and obtained via Laboratoire Sanoflore (Renens, Switzerland). BEO was characterized at the laboratory of Sanoflore using a Hewlett Packard GC-MS instrument with combined mass spectrometric and flame ionisation detectors together with an Agilent J&W HP-INNOWax polyethylene glycol (PEG) stationary phase (ID 0.25 mm; length 60 m; film 0.50 µm) and a temperature gradient stating with 6 min at 50°C, followed by an increase up to 250°C at a rate of 2°C per min, and ending with a second stable phase for 10 min at 250°C. Helium was chosen as vector gas and applied with a pressure of 23 psi. This measurement revealed the tested brand of BEO to be composed of 45.45% limonene, 23.10% linalyl acetate, 8.05% γ-terpinen, 7.25% β-pinene, 6.50% linalool, 1.35% α-pinene, and 0.35% geranial (a huge variety of unidentified minor compounds constitute the remaining 7.95%).

Psychoendocrinological Parameters

Salivary Cortisol Level: Cortisol, which is one of the hormones of the adrenal cortex, controls carbohydrate metabolism, fat metabolism, and protein metabolism. Although it is an essential hormone for the body, there are reports indicating that this hormone is related to psychological stress: When we feel stress, its concentration increases within a few minutes but only returns to the initial level after a few hours [20]. The isolated cortisol, which was measured in the present research project, spreads from the blood into urine and saliva within seconds, with its concentration exceeding more than 90% of the original concentration in the blood [20].

During each permutation of R, RW, and RWB, all in all 3 saliva samples were collected from each of the volunteers – 1 after R, 1 after RW, and 1 after RWB. Sample collection was performed as follows: All volunteers collected a saliva sample in a plastic tube exactly 90 s after completion of the respective setup. After collection, all samples were immediately stored at –20°C. After defrosting at 37°C, the samples were centrifuged at 3000 rpm for 15 min using a KUBOTA 7000 high-speed refrigerated centrifuge in order to remove impurities and mucus. Thereafter, CS levels were determined using the Salivary Cortisol EIA Kit (Salimetrics, USA) for the enzyme-linked immunosorbent assay (ELISA).

Heart Rate Variability: The heart rate (R-R interval) was measured with a GMS AC-301A instrument produced by GMS (Tokyo, Japan). Data were collected without interruption from the very beginning to the very end of the experiment, using the analysis software MemCalc for Windows version 1.2 (GMS, Tokyo, Japan) in maximum entropy mode for the analysis of heart rate variability by spectral analysis of R-R interval variability, calculating the high-frequency (HF) components (0.15–0.4 Hz) as an indicator of the activity of the parasympathetic system [21]. These indicator values were calculated individually as average values over the 15 min of each of the experimental setups as well as over the subsequent 10 min of rest.

The differences between HF values during setups and subsequent rests were also calculated. Furthermore, low-frequency (LF) components (0.04–0.15 Hz), normalized LF power (LFn.u.), normalized HF power (HFn.u.) as well as the LF/HF ratio as an indicator of the activity of sympathetic nervous system were recorded.

Psychological Measurements

In order to evaluate the psychological condition of the volunteers at this specific time, psychological tests were conducted as described below in a 10-min time frame directly after the end of each of the 3 measuring conditions. The used questionnaires were identical in all 3 conditions. The Profile of Mood State (POMS) assesses 6 mood subscales: tension-anxiety, depression, anger-hostility, vigor, fatigue, and confusion. High vigor scores reflect good mood or emotions, whilst in the other subscales low scores reflect good mood or emotions. The Total Mood Disturbance (TMD) score is computed by adding the 5 negative subscale scores (tension-anxiety (T-A), depression (D), anger-hostility (A-H), fatigue (F), and confusion (C)) and subtracting the vigour (V) score. In 1990, Yokoyama et al. [22] translated the 65-item scale POMS into Japanese (Kaneko Shobo Co., Tokyo, Japan), and demonstrated the reliability and validity of the Japanese version of POMS in Japanese participants. This Japanese version was used for the present study. The validated State-Trait Anxiety Inventory (STAI) [23, 24] was used to assess the response of the psychological stress values on aroma treatment (STAI state). The STAI state subscales contain 20 items with subscale scores ranging from 20 to 80. In this score, higher scores indicate increased anxiety. Using the Fatigue Self-Check List [25], which was put forward by the Ministry of Education, Culture, Sports, Science and Technology of Japan for gathering information on consumer needs, 10 items for mental fatigue and 10 items for physical fatigue (all in all 20 items) were evaluated by the volunteers themselves on a 5-step scale (0–4 points) to receive a numerical measurement of their subjective fatigue. The applied questionnaires were designed for repeated use in a short time and have already been used in the same way in several other clinical studies [26, 27].

Statistical Analysis

In order to understand if there is a statistically significant difference between the 3 conditions R, RW, and RWB, the one-way Repeated Measures Analysis of Variance (rANOVA) was used. When comparing R and RW, R and RWB, and RW and RWB, the multiple comparison analysis according to Bonferroni was applied. The differences were regarded as significant at $\geq 5\%$ and calculated using the SPSS version 15.0 statistics software. A comparison between the 3 groups (R, RW, RWB) was performed, and the null hypothesis constituted a plurality. The more commonly applied t-test, which is typically used for the comparison of 2 groups, could not be applied.

Results

Changes in the Salivary Cortisol Level between the R, RW, and RWB Setup

For the average CS levels of the 41 volunteers after the setups R, RW, and RWB, see table 2. In the rANOVA test, significant differences between the setups R, RW, and RWB could be found ($F = 6.090$, $p = 0.003$). In the subsequent multiple comparison, significant differences in CS levels could be detected comparing R + RW setup ($p = 0.049$) and R + RWB setup ($p = 0.004$). The average CS level decreased from the highest values at R, followed by RW, to the lowest values at RWB. It could be demonstrated that these differences were independent from the sequence in which the experiments were performed.

Heart Rate Variability

Performing a spectral analysis with the heart rate analysis software MemCalc, the HF component, which is an indicator for the function of the parasympathetic nervous system, and the LF/HF ratio, which is an indicator of the sympathetic nervous system, could be calculated (table 2). During the 15 min of measurement for each condition as well as during the 10 min of rest afterwards, average heart rate variability was calculated and compared between R, RW, and RWB. As far as HF is concerned, no significant differences could be detected in any condition during the 15 min of exposure. However, during the 10 min of rest, the comparison of the HF between the 3 setups showed significant differences ($F = 6.176$, $p = 0.003$). In the subsequent multiple comparisons, the HF values measured during the 10 min of rest after the RW and RWB setups were significantly different ($p = 0.026$). The average HF value decreased in the sequence RWB, RW, to R showing the lowest values. As far as the LF/HF ratio is concerned, no differences between any of the 3 setups could be found. The LF components measured during the 15 min of the setups did not display any significant effects in the subsequent multiple comparisons. However, during the last 10 min of the setups R and RWB significant effects ($p = 0.048$) could be observed.

For the other measured parameters, such as LFn.u., HFn.u., and LF/HF ratio, no significant differences between any of the 3 setups could be found.

Psychological Measurements

Immediately after each of the 3 setups was measured, the volunteers completed a questionnaire concerning their mood, feelings, anxiety, and fatigue. The results are shown in table 2.

POMS

When comparing the 3 measured setups, significant difference could be seen in the item scores T-A ($F = 3.863$, $p = 0.025$), A-H ($F = 3.238$, $p = 0.044$), V ($F = 17.838$, $p < 0.001$), F ($F = 21.389$, $p < 0.001$), C ($F = 6.479$, $p = 0.002$), and TMD ($F = 16.274$, $p < 0.001$). In the subsequent multiscale analysis, differences were found especially between the scores of the R and RWB setups for T-A ($p = 0.040$), V ($p < 0.001$), F ($p < 0.001$), C ($p = 0.008$), and TMD ($p < 0.001$) with the latter values indicating that negative mood of the study participants has decreased. Furthermore, in the case of V, a significant difference could also be detected between the RW and RWB condition ($p = 0.049$), demonstrating that the test subjects' vigor could indeed be significantly increased by the pharmacological action of the volatile oil as opposed to the effect of water vapor alone. There were also significant differences between the R and RW setups for the items V ($p = 0.001$), F ($p < 0.001$), and TMD ($p = 0.001$).

STAI

The STAI scores were different for all 3 measured setups ($F = 9.969$, $p < 0.001$). In the subsequent multiscale analysis, this difference was significant for both the comparisons of R and RWB ($p = 0.001$) as well as RW and RWB ($p < 0.001$). The average values decreased in the sequence from the highest values for R, to the lower RW, and finally the lowest RWB values.

Fatigue Self-Check List

Concerning both physical fatigue ($F = 3.124$, $p = 0.049$) and psychological fatigue ($F = 3.259$, $p = 0.044$), all 3 conditions gave diverting results. In the subsequent multiscale analysis, a significant difference between the R and RWB condition ($p = 0.041$) was detected. For both physical and psychological fatigue the values decreased from the highest values for R, to RW, and finally to the lowest values for RWB.

Side Effects

None of the study participants did report any kind of side effect, such as cough, during the present study.

Discussion

In a randomized crossover clinical trial, 41 healthy adult women were exposed to the 3 testing conditions R, RW, and RWB for

15 min each, elucidating significant differences between the 3 compared setups concerning CS, HF components of heart rate variability as well as psychological mood indicators, such as anxiety and fatigue. When inhaling BEO dissolved in water vapor, CS was significantly reduced and the HF component of the heart rate variability, which is an indicator for the function of the parasympathetic nervous system, significantly increased.

However, whilst an effect on the CS level could be shown, this difference was observed only between the R and RW setups as well as between the R and RWB setups, but unexpectedly not between the RW and RWB setups. Another surprising observation was that the HF

Table 2. Statistics for salivary cortisol, high frequency and low frequency/high frequency components, mood, anxiety, and fatigue after rest, rest + vapor, and rest + vapor + bergamot oil (n = 41)

Parameter		Mean ± SE			Repeated ANOVA		Bonferroni multiple test		
					F (R)	p value	p value		
		R	RW	RWB	(RW)	(RWB)	(R) - (RW)	(R) - (RWB)	(RW) - (RWB)
Cortisol	CS level	0.16 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	6.09	0.003*	0.049*	0.004*	n.s.
HF (ms ²)	setting a	630.92 ± 110.15	499.34 ± 61.51	589.13 ± 105.32	1.395	n.s.	n.s.	n.s.	n.s.
	setting b	600.27 ± 112.18	557.19 ± 91.58	534.76 ± 101.75	0.914	n.s.	n.s.	n.s.	n.s.
	rest a	475.71 ± 61.62	456.44 ± 56.50	540.94 ± 74.11	6.176	0.003*	n.s.	0.067	0.026*
	setting c	-37.66 ± 52.04	-35.64 ± 47.57	3.51 ± 72.16	0.822	n.s.	n.s.	n.s.	n.s.
	Setting d	-20.52 ± 52.04	-19.22 ± 47.57	-13.28 ± 72.16	0.028	n.s.	n.s.	n.s.	n.s.
HFn.u.	Setting a	28.87 ± 1.74	30.21 ± 2.12	30.23 ± 2.02	0.484	n.s.	n.s.	n.s.	n.s.
	Setting b	28.54 ± 1.77	29.99 ± 2.29	29.89 ± 1.94	0.248	n.s.	n.s.	n.s.	n.s.
	rest a	29.21 ± 2.10	27.95 ± 1.92	27.65 ± 1.88	0.619	n.s.	n.s.	n.s.	n.s.
	setting c	0.93 ± 1.51	-1.87 ± 1.68	-2.05 ± 1.40	1.154	n.s.	n.s.	n.s.	n.s.
	setting d	1.06 ± 1.58	-1.88 ± 1.76	-2.23 ± 1.43	1.229	n.s.	n.s.	n.s.	n.s.
LF (ms ²)	setting a	1242.33 ± 116.40	1213.50 ± 122.24	1109.86 ± 104.13	1.552	n.s.	n.s.	n.s.	n.s.
	setting b	1259.02 ± 116.46	1226.79 ± 133.75	1085.81 ± 104.54	1.027	n.s.	n.s.	0.048*	n.s.
	rest a	1122.48 ± 100.80	1158.82 ± 83.48	1141.16 ± 91.18	0.012	n.s.	n.s.	n.s.	n.s.
	setting c	-56.03 ± 101.47	-54.68 ± 90.15	31.30 ± 92.95	0.754	n.s.	n.s.	n.s.	n.s.
	setting d	-73.15 ± 103.19	-67.96 ± 101.02	55.35 ± 95.91	1.059	n.s.	n.s.	n.s.	n.s.
LFn.u.	setting a	71.13 ± 1.74	69.79 ± 2.12	69.03 ± 2.11	0.484	n.s.	n.s.	n.s.	n.s.
	setting b	71.46 ± 1.77	70.01 ± 2.29	70.11 ± 1.94	0.436	n.s.	n.s.	n.s.	n.s.
	rest a	70.79 ± 2.10	72.00 ± 1.92	71.99 ± 1.88	0.619	n.s.	n.s.	n.s.	n.s.
	setting c	-0.93 ± 1.51	2.21 ± 1.68	2.22 ± 1.40	1.154	n.s.	n.s.	n.s.	n.s.
	setting d	-1.06 ± 1.58	2.22 ± 1.76	2.40 ± 1.43	1.229	n.s.	n.s.	n.s.	n.s.
LF/HF	setting a	3.53 ± 0.41	3.43 ± 0.46	3.72 ± 0.63	0.177	n.s.	n.s.	n.s.	n.s.
	setting b	3.59 ± 0.37	3.79 ± 0.49	3.53 ± 0.43	0.200	n.s.	n.s.	n.s.	n.s.
	rest	3.60 ± 0.37	3.72 ± 0.36	3.66 ± 0.51	0.109	n.s.	n.s.	n.s.	n.s.
	setting a	-0.06 ± 0.24	0.12 ± 0.38	0.14 ± 0.31	0.097	n.s.	n.s.	n.s.	n.s.
	setting b	-0.16 ± 0.24	0.00 ± 0.44	0.12 ± 0.33	0.097	n.s.	n.s.	n.s.	n.s.
POMS (mood)	T-A	5.97 ± 0.65	4.90 ± 0.63	4.51 ± 0.56	3.863	0.025*	n.s.	0.040*	n.s.
	D	4.95 ± 1.19	4.09 ± 1.13	3.68 ± 1.01	2.339	0.097	n.s.	n.s.	n.s.
	A-H	1.68 ± 0.44	1.12 ± 0.32	0.78 ± 0.24	3.238	0.044*	n.s.	0.097	n.s.
	V	4.95 ± 0.61	8.14 ± 1.03	10.97 ± 0.90	17.838	<0.001*	0.001*	<0.001*	0.049*
	F	7.82 ± 0.73	4.78 ± 0.71	3.39 ± 0.55	21.389	<0.001*	<0.001*	<0.001*	n.s.
	C	6.60 ± 0.51	6.04 ± 0.54	5.14 ± 0.49	6.479	0.002*	n.s.	0.008*	n.s.
	TMD	22.10 ± 3.02	12.80 ± 3.39	6.53 ± 2.82	16.274	<0.001*	0.001*	<0.001*	n.s.
Anxiety	STAI	41.36 ± 1.19	37.12 ± 1.32	35.60 ± 1.32	9.969	<0.001	0.001	0.001	n.s.
Fatigue	body	4.04 ± 0.64	3.48 ± 0.50	3.07 ± 0.53	3.124	0.049	n.s.	0.041	n.s.
	mind	7.82 ± 0.97	7.75 ± 0.95	6.21 ± 0.79	3.259	0.044	n.s.	n.s.	n.s.
	body + mind	11.87 ± 1.54	11.24 ± 1.36	9.29 ± 1.20	3.571	0.033	n.s.	0.048	n.s.

*p < 0.050.
 Setting a: 15 min; setting b: 10 min, rest a: 10 min rest after setting; setting c: rest a – setting a; setting d: rest a – setting b.
 SE = standard error; R = rest; RW = rest + vapor; RWB = rest + vapor + bergamot oil; salivary cortisol; n.s. = not significant; HF = high frequency components; LF/HF = low frequency/high frequency components; HFn.u. = normalized HF power; LFn.u. = normalized LF power; POMS = Profile of Mood State; TMD = Total Mood Disturbance; STAI = State-Trait Anxiety Inventory.
 Negative subscale scores: T-A = tension-anxiety; D = depression; A-H = anger-hostility; V = vigor; F = fatigue; C = confusion.

values of the heart rate did not display significant differences among the 3 conditions during the 15 min of exposure but during the 10 min of rest after the conduction of each setup. Here, differences between the respective HF values could be detected, with the HF value after the application of BEO (RWB setup) being significantly higher than the R and RW setup. Similarly, although no significant effects on LF values were observed for the entire duration of any of the setups, LF values under the influence of BEO during the last 10 min of the RWB setup decreased significantly, indicating BEO to exert some influence also on the total power of the whole autonomic nervous system.

These unexpected observations can be explained by the experimental procedure which can be expected to result in a delay between inhaling BEO and the onset of its psychological and physiological effects. All in all, volatile oil can exert these effects on the HPA via 2 distinct ways: 1) After inhalation, it is transported into the nasal cavity, where it can act directly on the nervous system, entering the brain through the olfactory bulb. 2) The inhaled volatile oil components are transported into the alveolus of the lung, pass from there into the capillary blood stream, and are finally transported with the blood stream until they reach the nervous system and achieve their physiological effects on this second, independent way [28]. It is clear that while the first way acts directly on the brain and therefore facilitates very rapid outcomes, effects triggered via the second way occur with a substantial time lag.

In the present experiment, water and volatile oil were vaporized and diffused into the air, facilitating their inhalation into the lung in major quantities which are further transported through the alveoli into the blood, so that a pronounced effect can be expected. However, as these effects arose relatively slowly, this arrangement corresponds to the second above-mentioned pathway, which may explain why the significant effects on the HF values did not appear until the rest phase, after the experiment. The same is true for the CS measurements, where it might be possible that a significant difference between the values for RW and RWB could have been observed if the previously described experimental setups had been conducted over a longer time frame. Furthermore, when inhaling the volatile oil vapor, individual differences in respiration may also have had an effect on the observed results.

Still, the observed effects of BEO itself were quite impressive, with the HF values in the resting phase after the experiments being significantly improved in the RWB setup as compared to the RW setup with water vapor alone. The same is true for the V readings in the POMS which also improved significantly in RWB as compared to RW. The value of BEO in aromatherapy was further demonstrated by the scores for tension-anxiety (T-A), anger-hostility (A-H), and confusion (C) in the POMS as well as those for both physical fatigue and combined physical-mental fatigue in the Fatigue Self-Check List. All results for the RWB setup were significantly improved as compared to the R setup, while no significant difference could be found for any of these parameters comparing RW and R.

In a recent Taiwanese study [29], BEO was tested on adult volunteers in the form of a spray. After applying the spray for 10 min and subsequent 5 min of rest, the HF values increased significantly which is consistent with the results of the study at hand. Our find-

ings are also in good accordance with similar results observed for a combination of aromatherapy and other medicinal plants: In a current research project on 35 female subjects [30], inhaling the odor of saffron volatile oil for 20 min led to a decrease of both cortisol and anxiety level. In a most recent double-blind, randomized controlled trial [31], the effects of inhaling *Salvia sclarea* or *Lavandula angustifolia* essential oil with water vapor on systolic blood pressure, diastolic blood pressure, pulse rate, respiratory rate, and CS were investigated in 34 female patients with urinary incontinence. Here, the *S. sclarea* oil group experienced a significant decrease in systolic blood pressure as compared to the control; in contrast, the *L. angustifolia* aroma tended to increase systolic and diastolic blood pressure as compared to the control.

Among the identified individual constituents of BEO, limonene might be considered as a potential major contributor to the observed clinical effects as this predominant component (45.45 % of the essential oil) has been shown to significantly inhibit serotonin-induced locomotor activity [32].

Limitations

Due to the very concept of aromatherapy, a placebo control of the present human trial was not possible as the characteristic smell of BEO and the noticeable water vapor in the air made the difference between the R, RW, and RWB setup clearly apparent for all study participants. In this context it is debatable, how much the presence of smaller amounts of water vapor and BEO below the limit of human perception may influence the psychological and physiological parameters recorded in our present experiments. As the room was vented with an industrial fan between the individual setups until all participating experimenters unanimously judged the bergamot smell to have completely disappeared, we cannot rule out that trace amounts below the limit of human perception may have remained, influencing the subsequent experiments. It was for these reasons that we have examined all 6 possible permutations of the 3 setups with as many volunteers as our budget allowed. Fortunately, no significant influence of the sequence on the observed experimental outcomes was observed.

In view of these limitations of our present work, we are currently planning a follow-up study in a climate chamber for controlling the concentrations of BEO and water vapor in the air more strictly and in a longer time period for observing psychological and physiological effects that may only become apparent after a significant time lag. In this follow-up trial, a group of volunteers more representative of the general population – including male and older individuals – will be included in order to provide an even broader clinical basis for evaluation of BEO aromatherapy.

Conclusion

In the present study BEO was inhaled together with water vapor through the nose into the alveoli, inducing mental and physical effects by inhalation alone. It can therefore be used as a relatively simple form of stress reduction, which might be useful in our modern society plagued by chronic stress.

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Disclosure Statement

The authors declare that there is no conflict of interests concerning this paper.

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