Human Behavioral and Physiological Reactions to Inhalation of Sweet Orange Oil

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Abstract

Although essential oils are used increasingly for the improvement in quality of life as well as for the relief of various symptoms in patients, scientific evaluation of the effects of fragrances in healthy volunteers is rather scarce. Up to now, no experiments about the effects of sweet orange oil (Citrus sinensis) on human physiological parameters and on behavioral measures after inhalation have been carried out. Therefore, the main objective of the present study was to investigate the effects of this fragrance compound on physiological parameters as well as self-evaluation in healthy human subjects following inhalation. Physiological parameters recorded were blood pressure, breathing rate, skin temperature, and heart rate. Self-evaluation was assessed in terms of alertness, attentiveness, calmness, mood, relaxation, and vigour. Additionally, the fragrance was rated in terms of pleasantness, intensity, and effect. Sweet orange oil caused significant increases in heart rate as well as in subjective alertness, which are likely to represent a stimulating effect of the oil. These findings furnish scientific proof for the use of sweet orange oil in aromatherapy for the relief of mild forms of depression and stress in humans.

INTRODUCTION

Presently, the knowledge of the psychotherapeutic effects of fragrances has grown considerably. Especially in aromatherapy, Klemm et al. (1992) studied the human physiological responses to aromas. Their responses were assessed by EEG. All odors used affected the EEG in at least some subjects, and all subjects responded to at least some odors. Bursts of heliotropine (a vanilla-like scent) were administered to patients undergoing magnetic resonance imaging (MRI) and this reduced recalled anxiety by 63% in those who liked the smell (Redd et al., 1994). The effects of peppermint oil caused changes in brainwave patterns which were associated with alertness. In addition the peppermint aroma enhanced the sensory pathway for visual detection, which allowed the subjects more control over their allocation of attention (Parasuraman, 1991). Essential oils, i.e. Citrus lemon (lemon), Eucalyptus globulus (blue gum), Lavandula angustifolia (lavender), and Boswellia carteri (frankincense) were used for women with extensive severe burns (Price, 1989). For patients with Alzheimers, scented oils have the ability to act as a trigger and help them recapture some of their past experiences (Brett, 1997).

Although essential oils are used increasingly for the improvement of the quality of life as well as for the relief of various symptoms in patients, scientific evaluation of the effects of fragrances in healthy volunteers is rather scarce. Up to now, no experiments about the effects of sweet orange oil on human physiological parameters and on behavioral measures after inhalation have been carried out. Therefore, the main objective of the present study was to investigate the behavioral and physiological reactions to sweet orange oil in healthy human subjects following inhalation.
MATERIALS AND METHODS

Subjects and Fragrance Compounds

Twenty-four (12 males and 12 females) healthy volunteers between the ages of 19 and 23 years (mean age 21.38 ± 1.21 years) took part in the experiments. They were recruited by advertisement at Srinakharinwirot University. Subjects were tested in individual sessions and randomly assigned to either the control group or the sweet orange oil group. Each group consisted of 12 subjects (6 males and 6 females). Twenty-four hours prior to testing subjects had to abstain from food and beverage containing the substances tested, e.g. Oranges, Lemons, Ceylon cinnamon, Sassafras, Eucalyptus, Caraway, Fennel, Chewing gum, as well as from tea, coffee, and alcoholic beverages. Sweet orange oil (Citrus sinensis) was obtained from FPI Sale Ltd., Stamford, England. The oil contains up to 98% of the monoterpene limonene.

Fragrance Administration

The fragrance and a placebo substance were administered by inhalation via an aroma lamp, consisting of a candle and a small ceramic bowl. In the experimental group the small ceramic bowl was filled with the fragrance and pure water. In the control group it was filled with only pure water. 1.2 g sweet orange oil in 3.5 g pure water was used in the experimental group, while 5 g pure water was used in the control group.

Experimental Design

The experimental design is shown in Fig. 1. One session consisted of two trials of 20 minutes each. At the beginning and end of each trial subjective mental and emotional condition was assessed by visual analogue scales (VAS). Physiological parameters were recorded continuously during each trial. Blood pressure was measured at the beginning as well as at the end of each trial. In the first trial, which served as a control for influences of the experimental setup, the placebo substance was administered to all subjects. In the second trial the placebo was again administered to the control group, whereas in the experimental groups the appropriate fragrance was administered.

Acquisition of Physiological Parameters

Breathing rate (BR), heart rate (HR), and skin temperature (ST) were recorded simultaneously and in real time. All parameters were measured using Power Lab/4SP hardware (ADInstruments, Inc., NSW, Australia). Sampling rate was 100 Hz. Heart rate was assessed employing a Bio Amplifier (ML132) and a disposable ECG electrode (MLA1010). Skin temperature was measured by means of a Bridge Amplifier (ML110) and a skin temperature transducer (MLT409). Breathing rate was measured using a Bridge Amplifier (ML110) and the nasal temperature probe (MLT415) which registers breathing cycles on the basis of the difference in temperature between inhaled and exhaled air. Systolic and diastolic blood pressure (SBP and DBP) were measured in the dominant arm by sphygmomanometry using an automated system (Digital Electronic Model DS-155E, Japan). SBP and DBP were measured four times throughout the experiment.

Visual Analogue Scales (VAS)

VAS were used to assess subjective mental and emotional condition. They consisted of 100 mm lines for six items: relaxation, vigor, calmness, attentiveness, mood and alertness. Each subject was asked to mark his or her feeling for each item between the two possible extremes.

Procedure

All experiments were conducted in a bright and quiet room. Ambient temperature was 24-26°C. Upon arrival, the volunteers were interviewed about their personal data, i.e. name, age, sex, weight and height. In addition, they were asked about the rating of mental
and emotional condition. After completion of the interview and rating scales, SBP and DBP were measured. Subsequently, subjects were informed about the proceedings. Afterwards subjects were seated in a semi-reclined position, providing easy access to attach the electrodes. Electrodes were attached on the suitable positions. The fragrance or the placebo substance was administered as mentioned above. Next, the recording of the physiological parameters began. After completion of the first trial, subjects were asked to rate the rating scales. SBP and DBP were measured at the end of the first trial. This procedure was repeated in the second trial.

Data Reduction
The physiological recordings of each subject were computed trial by trial using Chart® software. Each trial was divided into 4 intervals of 5 minutes. Mean values of ST, and HR within each interval were obtained for each subject. BR was determined by counting the number of breaths per interval and was subsequently converted into breaths per minute. For each physiological parameter the difference scores between the first and the second trial were calculated. Additionally, for each subject difference scores between blood pressure measurements 2 and 4 were calculated.

For mental and emotional condition rating as well as fragrance ratings, on each scale the distance of the mark from the left-hand side was measured in mm. Individual difference scores between ratings 2 and rating 4 were calculated for each item.

Statistical Analysis
SPSS (1999) was used for data analysis. Taking into account the great inter-individual variability and the rather small sample size, only non-parametric test (i.e. Mann-Whitney-U-Test) was used in this study. The effects of fragrances on physiological parameters, ratings of mental and emotional condition, and odor ratings were determined by comparing the difference scores between the control group and the experimental group.

Correlational analyses were performed by means of Bravais-Pearson correlation and Spearman rank correlation. To evaluate correlations among physiological parameters the Bravais-Pearson correlation was carried out. Spearman rank-order correlation coefficient was used to analyze the relation between subjective ratings and physiological parameters.

RESULTS
Physiological Parameters
Mean difference scores and SEM of HR of the control group and the sweet orange oil group are shown in Fig. 2. HR of subjects in the control group only changed marginally in the second trial as compared to the first trial, resulting in a very small positive difference score. HR of subjects in the sweet orange oil group increased in the second trial as compared to the first trial, resulting in a negative difference score. Comparison of the difference scores revealed a significantly larger increase of HR in the sweet orange oil group than in the control group (P=0.037).

No significant effects of the sweet orange oil on BR, on ST, on DBP, and on SBP were found (p>0.1 for all, data not shown).

Mental and Emotional Conditions
Mean difference scores and SEM of alertness between rating 2 and rating 4 for the control group and the sweet orange oil group are shown in Fig. 3. Subjects in the control group felt more alert at the end of the second trial (rating 4) as compared to the end of the first trial (rating 2), resulting in a positive difference score. Subjects in the sweet orange oil group also rated themselves more alert at the end of the second trial as compared to the end of the first trial, resulting in a large positive difference score. Comparison of these difference scores (control vs. sweet orange oil) revealed a trend towards an increase of
subjective alertness in the sweet orange oil group ($P=0.083$).

No significant effects of sweet orange oil on subjective vigor, alertness, mood, relaxation, and calmness were found ($p>0.1$ for all, data not shown).

**Subjective Odor Ratings**

Mean difference scores and SEM of odor ratings (i.e. effect and intensity) for the control group and the sweet orange oil group are shown in Fig. 4.

Subjects in the control group rated the odor of the placebo (pure water) in the second trial hardly more stimulating than that of water in the first trial, resulting in a small positive difference score. In the experimental groups the odor of sweet orange oil in the second trial was rated more stimulating than that of water in the first trial, resulting in a positive difference score. Comparison of the difference scores only revealed a trend towards a larger stimulating effect of the odor of sweet orange oil than of the placebo substance ($p=0.074$).

Subjects in the control group rated the odor of the placebo (pure water) in the second trial hardly more intense than that of water in the first trial, resulting in a small positive difference score. In contrast, in the experimental group the odor of sweet orange oil in the second trial was rated as clearly more intense than that of water in the first trial, resulting in a significantly larger positive difference score ($p=0.002$).

No significant differences of odor pleasantness were found between groups ($p>0.1$ for all, data not shown).

**Correlational Analyses**

For each group correlational analyses were performed on the inter-trial difference scores of the physiological parameters, the difference scores between the second and the fourth measurement of SBP and DBP as well as the difference scores between the second and the fourth rating of the self evaluations. Spearman rank correlation coefficient ($\rho$) was used to analyze interactions between subjective ratings and physiological parameters. With a sample size of $N=12$, $\rho \geq |0.497|$ is considered to be statistically significant at the 0.05 level (Bortz et al., 1990). Pearson correlation coefficient ($r$) was used to detect interactions among the physiological parameters. Significant correlations are presented in Table 1.

In the control group changes of SBP were correlated with changes of DBP and HR: the more SBP increased, the more DBP and HR rose ($r=+0.646$, $P=0.023$ and $r=+0.590$, $P=0.044$, respectively). Also, a relation between changes of DBP and ST was revealed: the more DBP increased, the less ST rose ($r=-0.815$, $P=0.001$). Changes of subjective attentiveness were correlated with change of BR: the more attentive subjects rated themselves, the more BR rose ($\rho=-0.503$). Subjective evaluation of the fragrance’s effect and intensity interacted with changes of DBP and ST: the more stimulating the substance was rated, the less ST rose ($\rho=-0.533$); the more intense the substance was rated, the more DBP increased and the less ST increased ($\rho=+0.545$ and $-0.720$, respectively). Additionally, interactions between changes of subjective vigor and alertness were found: the more vigorous subjects felt, the more alert they judged themselves ($\rho=+0.567$). Interactions were revealed between subjective evaluation of the fragrance’s effect and changes in subjective vigor: the less stimulating the substance was rated, the more vigorous subjects felt ($\rho=+0.552$). Also, subjective rating of the fragrance’s effect was correlated with subjective rating of the intensity: the more stimulating the substance was rated, the more intense the substance was judged ($\rho=+0.575$).

In the sweet orange oil group, changes of BR were correlated with changes of ST: the more BR increased, the less ST rose ($r=-0.609$, $P=0.082$). Changes of subjective relaxation were correlated with changes of HR: the more relaxed subjects rated themselves, the less HR rose ($\rho=+0.743$). Additionally, subjective evaluation of the fragrance’s intensity interacted with changes of DBP: the more stimulating the substance was rated the less DBP increased ($\rho=0.560$). Moreover, interactions were revealed between subjective evaluation of the fragrance’s effect and changes in subjective
alertness: the more stimulating the substance was rated the more alert subjects felt ($\rho=+0.832$).

**DISCUSSION**

In the present investigation sweet orange oil was administered by inhalation to healthy subjects. Physiological parameters, i.e. blood pressure, heart rate, breathing rate, and skin temperature, were recorded as indicators of the arousal level of the autonomic nervous system. In addition, subjects had to rate their mental and emotional condition in terms of relaxation, vigor, calmness, attentiveness, mood, and alertness in order to assess subjective behavioral arousal.

Inhalation administration of sweet orange oil caused a significant increase of heart rate. Since heart rate is determined by the activity of the sympathetic branch of the ANS, an increase of heart rate shows an increase of sympathetic tone, i.e. an increase of physiological arousal. Thus, sweet orange oil may be characterized as physiologically stimulating. At the behavioral level, subjects in the sweet orange oil group rated themselves more alert than subjects in the control group. This finding points towards an increase of arousal in terms of self-evaluation.

The present investigation demonstrated that the effect of sweet orange oil is similar to that of its main component (i.e. limonene) which has been described by Heuberger (Heuberger et al., 2001). Their studies revealed that limonene led to an increase of blood pressure (i.e. an increase of ANS arousal) as well as subjective alertness and restlessness (i.e. an increase of subjective behavioral arousal). Therefore it may be speculated that the effect of sweet orange oil was caused by its main component limonene.

In conclusion our investigation showed a stimulating effect of the essential oil of sweet orange. These findings furnish scientific proof for the use of the sweet orange oil in aromatherapy for the relief of mild forms of depression and stress in humans.

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**Literature Cited**

Tables

Table 1. Correlational analysis for the control group (i.e. placebo) and the experimental group (i.e. sweet orange oil).

<table>
<thead>
<tr>
<th>Substance</th>
<th>PHY¹/PHY¹</th>
<th>PHY¹/SE²</th>
<th>PHY¹/FR³</th>
<th>SE²/SE²</th>
<th>FR³/SE²</th>
<th>FR³/FR³</th>
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<tbody>
<tr>
<td>Placebo</td>
<td>SBP↑/DBP↑</td>
<td>BR↑/AT↑</td>
<td>ST↓/E↑</td>
<td>V↑/AL↑</td>
<td>E↓/V↑</td>
<td>E↑/I↑</td>
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<tr>
<td></td>
<td>SBP↑/HR↑</td>
<td>DBP↑/I↑</td>
<td>ST↓/I↑</td>
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<td></td>
</tr>
<tr>
<td>Sweet orange oil</td>
<td>BR↑/ST↓</td>
<td>HR↓/R↑</td>
<td>DBP↓/I↑</td>
<td>E↑/AL↑</td>
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</tbody>
</table>

¹ PHY: physiological parameter, HR: heart rate, BR: breathing rate, ST: skin temperature, ↑/↓: increase/decrease in trial 2 as compared with trial 1, SBP: systolic blood pressure, DBP: diastolic blood pressure, ↑/↓: increase/decrease on measurement 4 as compared with measurement 2.


³ FR: fragrance rating, E: (stimulating) effect, I: intensity, P: pleasantness, ↑/↓: increase/decrease of rated odor quality in rating 2 as compared with rating 1, ↑/↓: increase/decrease.

Figures

Fig. 1. Experimental design.

Fig. 2. Mean difference scores and SEM of heart rate for the control group (i.e. placebo) and the experimental group (i.e. sweet orange oil): p=0.037.
Fig. 3. Mean difference scores and SEM of subjective alertness for the control group (i.e. placebo) and the experimental group (i.e. sweet orange oil): p=0.083.

Fig. 4. Mean difference scores and SEM of subjective odor ratings for the control group (i.e. placebo) and the experimental group (i.e. sweet orange oil): odor effect, p=0.074; odor intensity, p=0.002.