Evaluation of the Effects of East Indian Sandalwood Oil and α-Santalol on Humans after Transdermal Absorption

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Original Paper

Abstract

The aim of the study was to investigate the effects of East Indian sandalwood oil (*Santalum album*, Santalaceae) and α-santalol on physiological parameters as well as on mental and emotional conditions in healthy human subjects after transdermal absorption. In order to exclude any olfactory stimulation, the inhalation of the fragrances was prevented by breathing masks. Eight physiological parameters, i.e., blood oxygen saturation, blood pressure, breathing rate, eye-blink rate, pulse rate, skin conductance, skin temperature, and surface electromyogram were recorded. Subjective mental and emotional condition was assessed by means of rating scales. While α-santalol caused significant physiological changes which are interpreted in terms of a relaxing/sedative effect, sandalwood oil provoked physiological deactivation but behavioral activation. These findings are likely to represent an uncoupling of physiological and behavioral arousal processes by sandalwood oil.

Key words
Autonomic nervous system · human · sandalwood · α-santalol · subjective evaluation · transdermal administration

Introduction

The essential oil of East Indian sandalwood (*Santalum album*, Santalaceae) has a long history of use in oriental medicine. The interest in the usage of sandalwood oil and one of its main compounds, α-santalol, as therapeutic substances has grown considerably. The oil has been associated with chemopreventive activity since it has been found to enhance the activity of glutathione *S*-transferase (GST) and to increase the level of acid-soluble sulfhydryl groups (SH) in mice [1]. Antiviral activity of sandalwood oil has been demonstrated by Benencia et al. [2]. Okugawa et al. [3] described the effects of α- and β-santalol on the central nervous system. Their results showed that both fragrances could be considered as neuroleptics with some resemblance to the pharmacological activity of chlorpromazine. In recent years, the use of fragrances for clinical purposes has been propagated [4]. A study conducted at the Royal Sussex County Hospital [5] showed that foot massage with the essential oil of lavender lowered blood pressure as well as heart and respiratory rates of the patients in an intensive care unit. In a similar investigation at the Middlesex Hospital in London [6] intensive care patients were given foot massage using the essential oil of *Citrus aurantium* (Neroli oil). The results suggested that massage with essential oils positively affects the psychological state of the patients. Topical application of essential oils in a carrier lotion has been reported by Macdonald [7]. This study demonstrated the enhancement of conventional methods of arthritic pain relief by the usage of essential oils. Although massage of essential oils is used growingly for the improvement of the quality of life as well as for the relief of various symptoms in patients, scientific evaluations of the effects of transdermal administration of fragrances in healthy volunteers are rather scarce. Up to now, no experiments about the effects of sandalwood oil and one of its main components, α-santalol, on human physiological parameters and

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on behavioral measures after percutaneous administration have been carried out. Therefore, the main objective of the present study was to investigate the effects of these fragrance compounds on parameters of the autonomic nervous system as well as on mental and emotional conditions in healthy human subjects following transdermal absorption.

Materials and Methods

Subjects and fragrance compounds

Thirty-six healthy volunteers aged between 19 and 32 years (mean age 23.18 ± 2.64 years) took part in the experiments. Subjects were tested in individual sessions and randomly assigned to either the control group or one of two experimental groups, i. e., a sandalwood oil group and an α-santalol group. Each group consisted of 12 subjects. They were fully briefed, gave written informed consent to all aspects of the study (Viennese ethic commission's permissions No. 324/96, 419/98) and were free to withdraw at any time. East Indian sandalwood oil (commercially available from Dragoco GmbH, Holzminden, Germany, product No. 16 598) and the main component α-santalol were used as fragrances in this study. East Indian sandalwood oil was identified by Dr. Leo Jirovetz, Department of Pharmaceutical Chemistry, University of Vienna. The most prominent constituents are santalols (90%). A voucher specimen (No. 4/942 130) is deposited in the refrigerator (4 °C) at room number 2E464 of that department. α-Santalol was separated from a mixture of α- and β-santalols (Sigma-Aldrich Chemie GmbH, D-89 555 Steinheim, Germany, CAS 11 031-45-1) via argentation column chromatography [8]. Complete identification of each obtained santalol was accomplished by ^{1}H- and ^{13}C-NMR-spectroscopy and GC-MS, and comparison of these data with those in the literature [3].

Fragrance administration

In the experimental groups, 20% (w/w) solutions of either sandalwood oil or α-santalol in peanut oil were used. One mL of this solution was applied to the skin of the lower abdomen of each subject. In the control group, 1 mL of the placebo substance, i. e., pure peanut oil, was used. In all groups subjects were supplied with pure air by breathing masks in order to prevent any olfactory stimulation.

Experimental design

The experimental design is shown in Fig. **1**. One session consisted of two trials of 20 minutes each. At the beginning as well as at the end of each trial, subjective mental and emotional condition was assessed by visual analogue scales (VAS). Physiological parameters were recorded continuously during each trial. In the first trial, which served as a control for influences of the experimental set-up, 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

Blood oxygen saturation (BOS), breathing rate (BR), eye-blink rate (EBR), pulse rate (PR), skin conductance (SC), skin temperature (ST), and surface electromyogram (EMG) were recorded simultaneously and in real time on the non-dominant side of the body. All parameters were measured using MP100WSW hardware (Biopac Systems, Inc., Santa Barbara, California, USA) including sensors and Ag/AgCl surface electrodes and Acq*knowledge*® software (V3.2.6, © 1992 – 1997, Biopac Systems, Inc., Santa Barbara, California, USA). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the dominant arm by sphygmomanometry using an automated system (Hartmann Digital HG160, Paul Hartmann AG, D-89522 Heidenheim). Details of the recording system and procedure have been described elsewhere [17].

Visual analogue scales (VAS)

VAS were used to assess subjective mental and emotional conditions. 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 21 – 24 °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. The electrodes and sensors were attached to the suitable positions. The breathing mask was attached to the subject's face to cover nose and mouth. Following THAT, subjects applied 1 ml of the placebo substance to the skin of their lower abdomen by themselves for approximately 2 – 3 minutes. The area was then covered with plastic film. Then, the recording of physiological parameters was started. After completion of the first trial the rating scales were presented. 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 Acq*Knowledge*® software. For each subject and

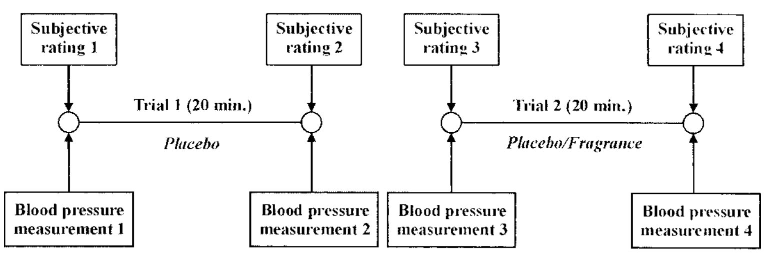

Fig. **1** Experimental design.

every parameter the mean value in the second trial was subtracted from the mean value in the first trial to give the individual inter-trial difference score. Additionally, for each subject difference scores between blood pressure measurements 2 and 4 were calculated. For subjective 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

Systat 9.0 (SPSS Inc., 1999) was used for data analysis. Mann-Whitney U test and Kruskal-Wallis one-way analysis of variances were used in this study. The effects of fragrances on physiological parameters and ratings of mental and emotional condition were determined by comparing the difference scores between the control group and the experimental groups. Furthermore, the difference scores of the sandalwood oil group were compared to those of the α-santalol group in order to detect differences in the effectiveness of the fragrances.

Correlational analyses were performed by means of Bravais-Pearson correlation and Spearman rank-order 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 and SEM of physiological parameters of the control group and the experimental groups are presented in Table **1**. Mean difference scores of PR of the control group and the experimental groups are shown in Fig. **2**. Comparison of the difference scores revealed a significantly larger decrease of PR in the α-santalol group than in the control group (P = 0.050). Mean difference scores of EBR of the control group and the experimental groups are shown in Fig. **3**. The difference scores of the sandalwood oil group and the α-santalol group were in significant contrast to that of the control group (control versus sandalwood oil: P = 0.025; control versus α-santalol: P = 0.033). Mean difference scores of SBP between measurement 2 and 4 for the control group and the experimental groups are shown in Fig. **4**. The difference scores of the sandalwood oil group and the α-santalol group were in marginal contrast to that of the control group (control versus sandalwood: P = 0.073; control versus α-santalol: P = 0.093). No significant effects of the sandalwood oil or α-santalol on BR, on SCL, on ST, on EMG, DBP, and on BOS were found (p > 0.1 for all, data not shown).

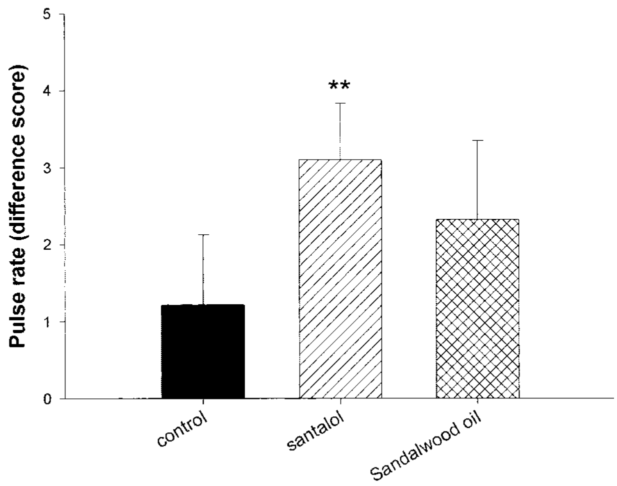

Fig. **2** Mean difference scores and SEM of pulse rate for the control group, the sandalwood oil group, and the α-santalol group. Asterisks (**) on the top of the error bars indicate significant differences ($P \leq$ 0.050) between the experimental groups and the control group.

Mental and emotional conditions

Mean difference scores of attentiveness between rating 2 and rating 4 for the control group and the experimental groups are shown in Fig. **5**. Comparison of these difference scores (control versus sandalwood oil) revealed a trend towards an increase of subjective attentiveness in the sandalwood oil group (P = 0.088). No significant effects of sandalwood oil or α-santalol on subjective

Table **1** Mean and SEM of physiological parameters of the control group and the experimental groups

			PR	*SBP*	*EBR*
C	trial 1	Mean (SEM)	67.737 (2.801)	117.167 (2.330)	18.916 (2.897)
	trial 2	Mean (SEM)	66.526 (2.529)	120.000 (2.610)	21.183 (3.322)
SAN	trial 1	Mean (SEM)	66.686 (2.742)	119.333 (2.520)	19.350 (4.003)
	trial 2	Mean (SEM)	64.369 (2.398)	117.667 (2.970)	18.713 (3.560)
α-SAN	trial 1	Mean (SEM)	72.381 (2.643)	128.750 (3.130)	13.203 (2.713)
	trial 2	Mean (SEM)	69.278 (2.516)	128.000 (3.520)	11.572 (2.556)

C: control group, SAN: Sandalwood oil group, α-SAN: α-santalol group; PR: pulse rate, SBP: systolic blood pressure, EBR: eye-blink rate.

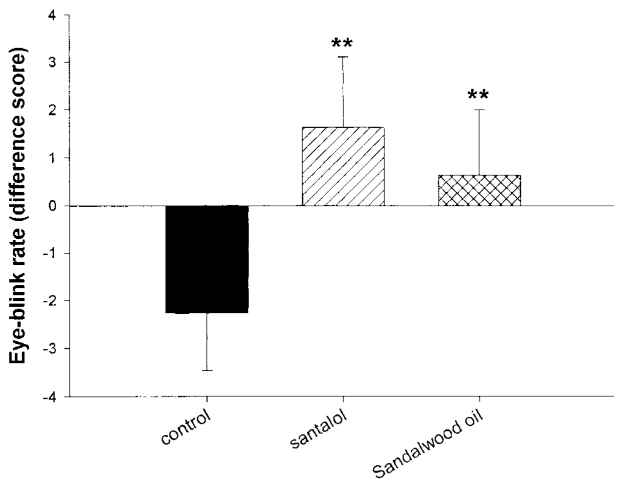

Fig. **3** Mean difference scores and SEM of eye-blink rate for the control group, the sandalwood oil group, and the α-santalol group. Asterisks (**) on the top of the error bars indicate significant differences ($P \leq$ 0.050) between the experimental groups and the control group.

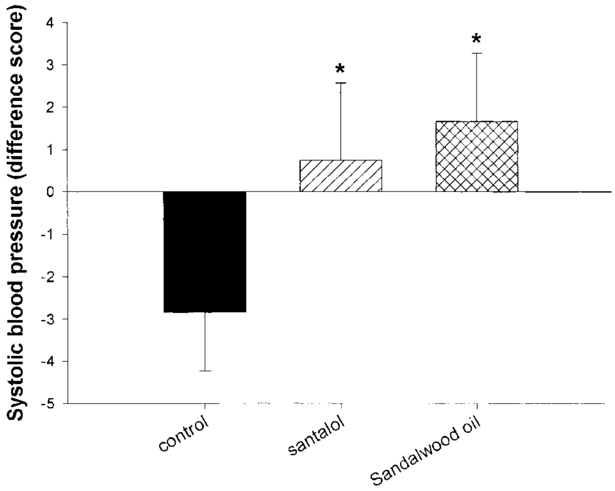

Fig. 4 Mean difference scores and SEM of systolic blood pressure for the control group, the sandalwood oil group, and the α-santalol group. Asterisks (*) on the top of the bars indicate significant differences (0.050 τ; $P \leq 0.100$) between the experimental groups and the control group.

vigor, alertness, mood, relaxation, and calmness were found (p > 0.1 for all, data not shown).

Correlations

In the control group changes of subjective attentiveness were correlated with changes of PR and SBP: the more attentive subjects rated themselves, the more PR and SBP rose (ρ = –0.734 and –0.591, respectively). Also, a relation between changes of DBP and SCL was revealed: the more DBP increased, the less SCL rose (r = –0.628, P = 0.029). Additionally, interactions between changes of subjective calmness, attentiveness, and relaxation were found: the more calm subjects felt, the more relaxed and the less attentive they judged themselves (ρ = + 0.733 and –0.704, respectively). Moreover, changes of subjective alertness interacted with changes of subjective vigor: the more alert subjects felt, the more vigorous they rated themselves (ρ = + 0.746).

In the sandalwood oil group changes of subjective attentiveness were correlated with changes of SBP: the more attentive subjects rated themselves, the less SBP rose (ρ = + 0.536). Additionally, interactions between changes of subjective calmness and relaxation were found: the more calm subjects felt, the more relaxed they felt (ρ = + 0.755). Moreover, changes of subjective alertness interacted with changes of subjective mood: the more alert subjects felt, the more cheerful they rated themselves (ρ = + 0.663).

In the α-santalol group relations between changes of SBP, SCL and PR were revealed: the less SBP increased, the less SCL rose (r = + 0.806, P = 0.002); the less PR increased, the less SCL rose (r = + 0.597, P = 0.041). Additionally, interactions between changes of subjective vigor, attentiveness, and alertness were found: the more vigorous subjects felt, the more alert and attentive they judged themselves (ρ = + 0.620 and + 0.732, respectively); the more alert subjects felt, the more attentive they rated themselves (ρ = + 0.524).

Discussion

In the present investigation essential sandalwood oil and one of its main components α-santalol were administered transdermally to healthy subjects. Inhalation of the fragrances was prevented by breathing masks in order to eliminate effects of subjective odor evaluation. Physiological parameters, i.e., blood oxygen saturation, blood pressure, eye-blink rate, pulse rate, breathing rate, skin conductance, skin temperature, and surface electromyogram, 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. Transdermal absorption of sandalwood oil led to a significant decrease of eye-blink rate and a trend towards a larger decrease of systolic blood pressure. Generally, eye movement and blink rate are indicative of cognitive processing and the level of arousal [9]. Therefore, the decrease of blink rate may be interpreted as a decrease of arousal. Since blood pressure is determined by the activity of the sympathetic branch of the ANS a decrease of systolic blood pressure shows a decrease of sympathetic tone, i.e., a decrease of physiological arousal. At the behavioral level, subjects in the sandalwood oil group rated themselves more attentive than subjects in the control group. This finding points towards an increase of arousal in terms of self-evaluation. Correlational analyses showed that changes of subjective attentiveness were correlated with changes of systolic blood pressure in both groups. The more systolic blood pressure decreased in subjects in the control group the less attentive they rated themselves, i.e., in this group a decrease of physiological arousal was correlated with a decrease in behavioral activation. In contrast, the more systolic blood pressure decreased in subjects in the sandalwood oil group the more attentive they judged themselves. Obviously, in the latter group a decrease of physiological activation was correlated with an increase of arousal at the subjective level. This finding may indicate that massage of essential sandalwood oil results in the uncoupling of physiological and behavioral arousal processes [10].

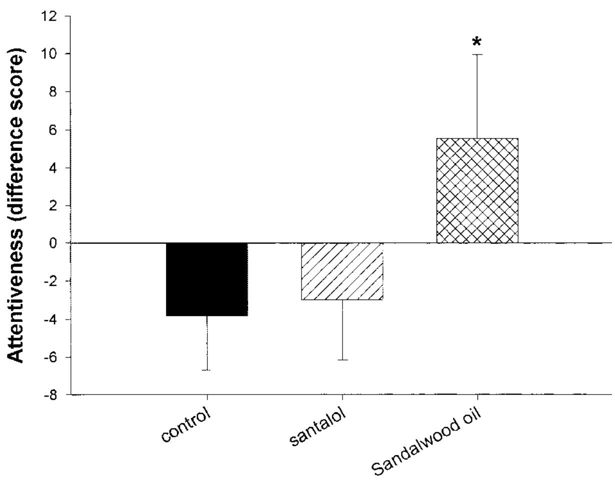

Fig. 5 Mean difference scores and SEM of subjective attentiveness for the control group, the sandalwood oil group, and the α-santalol group. Asterisks (*) on the top of the bars indicate significant differences (0.050 τ; $P \leq 0.100$) between the experimental groups and the control group.

The observed effects of essential sandalwood oil are not precisely characterized by concepts like relaxation or sedation, since deactivation on both the physiological level and on the level of self-evaluation is associated with these concepts. Massage of essential sandalwood oil, however, reduced the level of arousal of the autonomic nervous system but did not lead to deactivation at the behavioral level, i.e. after the administration of the oil subjects did not feel more relaxed or drowsy, but in contrast reported to feel more attentive than before the application of the oil. Thus, the effects of essential sandalwood oil may be characterized by the concept of "harmonization" rather than relaxation/sedation which has also been described for the essential oil of lavender [11], [12]. The decrease of eye-blink rate was not correlated with changes in self-evaluation. This finding suggests the effectiveness of pharmacological mechanisms, e.g., direct interactions between fragrance molecules and receptor sites which are involved in the regulation of ANS arousal. Due to their high lipophilicity fragrance molecules easily penetrate the blood-brain barrier [13], and animal experiments have shown that they interact with various receptor types in the brain, e.g., $GABA_A$ [14], glutamate [15], D_2 and $5\text{-}HT_{2A}$ [16]. Therefore, it seems likely that essential sandalwood oil exerts its effects by an interaction with central (e.g., hypothalamic, limbic) structures which control the level of physiological and/or behavioral arousal. Transdermal administration of α-santalol, like essential sandalwood oil, provoked a significant decrease of eye-blink rate which represents a decrease of arousal. In addition, α-santalol caused a significant decrease of pulse rate. Since pulse rate is mainly controlled by the parasympathetic nervous system the decrease of pulse rate after percutaneous absorption of α-santalol is likely to show an increase in vagal tone, i.e., a decrease of ANS arousal. As in the sandalwood oil group, a trend towards a larger decrease of systolic blood pressure in subjects in the α-santalol group as compared with subjects in the control group was revealed which again indicates a decrease of physiological arousal. Thus, α-santalol may be characterized as physiologically relaxing. However, administration of α-santalol had no effects on subjective mental and emotional conditions. Moreover, changes of physiological parameters were not correlated to changes of subjective mental and emotional conditions. It may again be speculated that the observed effects of α-santalol on the physiological parameters occur via pharmacological mechanisms.

In conclusion, our investigation showed "harmonizing" effects of the essential oil of East Indian sandalwood and relaxing/sedative effects of one of its main compounds, α-santalol. In addition, the present study showed that transdermal administration offers the opportunity to exclude psychological mechanisms that are active when odorous substances are applied by means of inhalation [17], [18] and to separately investigate effects of fragrances mediated by pharmacological mechanisms.

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