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One increasingly popular type of alternative therapy is aromatherapy, but scientific validation in this field is still rare. The aim of this study was to investigate the effect of rose oil (*Rosa damascena* Mill, Rosaceae) on human autonomic parameters and emotional responses in healthy subjects after transdermal absorption. In order to exclude any olfactory stimulation the inhalation of the fragrances was prevented by breathing masks. Forty healthy volunteers participated in the experiments. Five autonomic parameters, i.e. blood pressure, breathing rate, blood oxygen saturation, pulse rate, and skin temperature, were recorded. Emotional responses were assessed by means of rating scales. Compared to placebo, rose oil caused significant decreases of breathing rate, blood oxygen saturation and systolic blood pressure, which indicate a decrease of autonomic arousal. At the emotional level, subjects in the rose oil group rated themselves as more calm, more relaxed and less alert than subjects in the control group. These findings are likely to represent a relaxing effect of the rose oil and provide some evidence for the use of rose oil in aromatherapy, such as causing relief of depression and stress in humans.

Keywords: *Rosa damascena* massage aromatherapy, autonomic nervous system, relaxing effect.

Massage of essential oils is increasingly being used for the improvement of the quality of life, as well as for the relief of various symptoms in patients [1], but scientific evaluation of the effects of fragrances in healthy volunteers is rather scarce. Many researchers have attempted to prove the scientific effects of aromatherapy, but most of the aromatherapy trials were not controlled studies and their results are, therefore, possibly biased and not scientific. Presently, there are a variety of approaches to evaluate the physiological and psychological effects of fragrances such as measuring changes in autonomic parameters, for example heart rate, breathing rate, blood pressure, eye-blinks, skin temperature and skin conductance [2a-2c], changes in brain wave activities, for example electroencephalogram, contingent negative variation [2d-2f], changes in mood, cognitive performances and emotion [3].

In medicine, interest in the use of rose oil (*Rosa damascena* Mill, Rosaceae) as a therapeutically active agent has grown considerably. Especially in aromatherapy, rose essential oil has a soothing effect on the emotions and in particular, depression, apathy and grief. It is used for insomnia, headache, migraine, nervous tension and stress-related emotion [4a,4b]. In addition, rose oil reportedly helps to improve memory and brings a feeling of well-being. Recently antimicrobial activity of the rose oil has been reported [4c]. In animal studies, rose oil showed anti-conflict [4d,4e] and anxiolytic effects [4f,4g]. Furthermore, rose oil and its main components, citronellol and geraniol, had a spasmolytic effect on Guinea-pig ileum *in vitro* [4a]. Rose oil inhalation significantly prolonged the pentobarbital-induced sleeping time in rat [4h]. In humans, rose oil demonstrated a 40% reduction in relative sympathetic nerve activity and a 30% reduction in plasma adrenaline concentration [4i].

Although *R. damascena* essential oil is quoted extensively in the literature as being used for anxiolytic treatment, there have been relatively few published controlled studies of its efficacy in either decreasing anxiety or increasing relaxation. Up to now, no experiments on the effects of rose oil on human autonomic parameters and on emotional responses after transdermal administration have been carried out. Therefore, the main objective of the present study was to investigate the effects of this fragrance on autonomic parameters, as well as on emotional responses in healthy humans following transdermal absorption.

Table 1: Mean and SEM of autonomic parameters of the control group and the experimental group.

Autonomic parameters	Control (Mean±SEM)		Rose (Mean±SEM)	
	Trial 1	Trial 2	Trial 1	Trial 2
SBP	117.7±3.8	118.5±4.2	117.7±2.5	113.8±1.9
DBP	64.8±1.1	67.9±1.4	65.8±2.3	67.6±2.4
ST	33.9±0.2	33.9±0.3	33.8±0.3	34.9±0.3
BR	16.8±0.7	17.0±0.7	16.8±0.7	15.5±0.8
PR	66.8±2.6	63.9±2.5	64.7±1.7	64.3±1.8
BOS	98.0±0.3	98.2±0.3	98.1±0.2	97.0±0.2

SBP: systolic blood pressure DBP: diastolic blood pressure, ST: skin temperature, BR: breathing rate, PR: pulse rate, BOS: blood oxygen saturation, SEM: standard error mean

In the present investigation rose oil was administered transdermally to healthy subjects. Autonomic parameters, i.e. systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse rate (PR), blood oxygen saturation (BOS), breathing rate (BR), and skin temperature (ST), 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.

Autonomic parameters: The mean and SEM of autonomic parameters of the control group and the experimental group are presented in Table 1. SBP of subjects in the control group increased at the end of the second trial compared with the end of the first. In contrast, SBP of subjects in the rose oil group decreased at the end of the second trial compared with the end of the first. The difference scores of SBP between the second and first trials for the control group and the rose oil group are shown in Figure 1. Comparison of these difference scores revealed a significantly larger decrease of SBP in the rose oil group than in the control group (P=0.036). The rose oil group showed a significant decrease of blood pressure. Since blood pressure is determined by the activity of the sympathetic branch of the ANS, a decrease in blood pressure shows a decrease in sympathetic tone, i.e., a decrease of autonomic arousal [5].

ST of subjects in the control group only marginally changed in the second trial compared with the first one. In contrast, ST of subjects in the rose oil group increased in the second trial compared with the first. The different scores of ST between the second and first trials for the control group and the rose oil group are shown in Figure 1. Comparison of these scores revealed a significantly larger increase of ST in the rose oil group than in the control group (P=0.001). A significantly larger increase of skin temperature in the rose oil group compared with the control group was found. Skin temperature is controlled indirectly by the sympathetic division of the ANS via the contraction or relaxation of the smooth muscles which surround the blood vessels and regulate blood supply to distinct skin areas. When these muscles are contracted skin temperature is lower because less blood reaches there. On the other hand, when these muscles are relaxed, skin temperature is higher because more blood is supplied there. Therefore, the increase of skin temperature in the rose oil group indicates a decrease of ANS arousal [5].

BR of subjects in the control group increased at the end of the second trial compared with the end of the first one. In contrast, BR of subjects in the rose oil group decreased at the end of the second trial compared with the end of the first. The difference scores of BR between the second trial and the first trial for the control group and the rose oil group are shown in Figure 1. Comparison of these scores revealed a significantly larger decrease of BR in the rose oil group than in the control group (P=0.031). BOS of subjects in the control group only marginally changed in the second trial compared with the first one. In contrast, BOS of subjects in the rose oil group decreased in the second trial compared with the first. The difference scores of BOS between the second and first trials for the control group and the rose oil group are shown in Figure 1. Comparison of these difference scores revealed a significantly larger decrease of BOS in the rose oil group than in the control group (P=0.029). Transdermal absorption of rose oil led to significant decreases of breathing rate and blood oxygen saturation. In general, the cardiovascular system has a relationship with the respiratory system. Muscle sympathetic nerve activity is associated with respiratory function, namely, reduction in respiratory rate leads to a decrease of muscle sympathetic activity [6a]. Furthermore, a decrease in breathing rate may cause an increase of baroreceptor sensitivity and a decrease of blood oxygen saturation [6b]. No significant effects of the rose oil on DBP and on PR were found (P>0.05 for all).

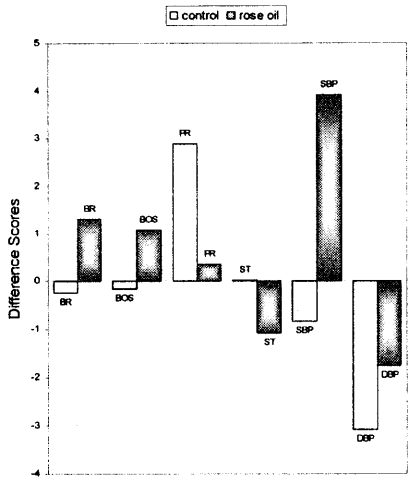

Figure 1: The difference scores of breathing rate (BR), blood oxygen saturation (BOS), pulse rate (PR), skin temperature (ST), systolic blood pressure (SBP) and diastolic blood pressure (DBP) for the control group and the rose oil group.

Emotional parameters: The mean and SEM of emotional parameters of the control group and the experimental group are presented in Table 2. Subjects in the control and the rose oil groups felt more alert at the end of the second trial compared with the end of the first. The difference scores of subjective alertness between the second and first trials for the control group and the rose oil group are shown in Figure 2. Comparison of these difference scores revealed a significant decrease of subjective alertness in the rose oil group compared with the control group (P=0.036). Subjects in the rose oil group rated themselves less alert than subjects in the control group. This finding points towards a decrease of arousal in terms of self-evaluation [5].

In addition, subjects in the control group felt less calm at the end of the second trial compared with the end of the first one. On the other hand, subjects in the rose oil group judged themselves more calm at the end of the second trial compared with the end of the first. The difference scores of subjective calmness between the second and first trials for the control group and the rose oil group are shown in Figure 2. Comparison of these difference scores revealed a significant increase of subjective calmness in the rose oil group compared with the control group (P=0.036). Subjects in the rose oil group rated themselves as more calm than subjects in the control group. This finding points towards a decrease of arousal in terms of self-evaluation [5]. Furthermore, subjects in the control and rose oil groups felt more relaxed at the end of the second trial compared with the end of the first trial.

Table 2: Mean and SEM of emotional parameters of the control group and the experimental group.

	Control (Mean±SEM)		Rose (Mean±SEM)	
	Trial 1	Trial 2	Trial 1	Trial 2
AT	14.8±2.6	10.1±2.3	23.6±3.4	17.0±3.1
AL	28.3±3.5	14.0±3.2	30.5±4.7	27.1±5.1
C	11.6±3.0	13.6±4.2	20.2±4.1	11.3±2.8
R	20.5±4.7	13.9±4.9	29.0±4.1	16.5±3.2
M	19.9±3.1	16.2±3.3	27.6±3.7	21.0±3.4
V	30.1±3.3	22.6±4.2	39.3±4.0	29.2±4.1

AT: attentiveness, AL: alertness, C: calmness, R: relaxation, M: mood, V: vigor.

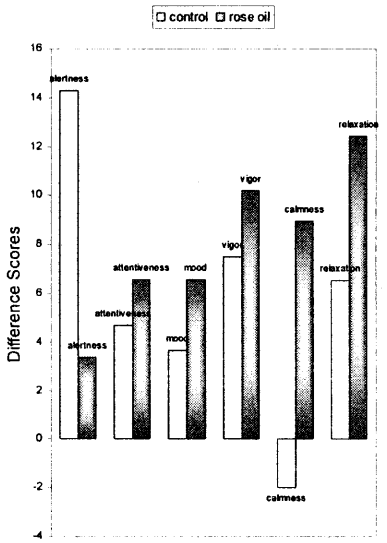

Figure 2: The difference scores of subjective alertness, attentiveness, mood, vigor, calmness, and relaxation for the control group and the rose oil group.

The difference scores of subjective relaxation between the second and first trials for the control and rose oil groups are shown in Figure 2. Comparison of these difference scores revealed a significant increase of subjective relaxation in the rose oil group compared with the control (P=0.027). Subjects in the rose oil group rated themselves more relaxed than subjects in the control group. This finding points towards a decrease of arousal in terms of self-evaluation [5]. No significant effects of the rose oil on subjective attentiveness, mood and vigor were found (P>0.05 for all).

Transdermal absorption of rose oil reduced the level of arousal of the autonomic nervous system (ANS), i.e. decreases of systolic blood pressure, breathing rate, blood oxygen saturation, and an increase in skin temperature. Moreover, massage of rose oil led to deactivation at the behavioral level, i.e. subjects feel more calm, more relaxed and less alert than before the administration of the oil. This finding points towards a decrease of arousal in terms of self-evaluation. Thus, the effects of rose oil by means of percutaneous administration may be characterized by

the concept of relaxation, which has also been described for sandalwood [6c] and ylang ylang (*Cananga odorata*) essential oils [6d].

Haze *et al.* [4i] described that both citronellol and geraniol, the main components of rose oil, induced inhibition of sympathetic activity. In addition, essential oils with a deactivating effect on the sympathetic activity, for example rose oil and patchouli oil, have been reported [4i]. These essential oils consist of alcohols such as citronellol, geraniol, nerol and patchouli alcohol, which are possibly the components that mediate the deactivating effect on sympathetic activity. Moreover, high concentrations of geraniol have estrogenic activity [6e], and estrogens have been shown to reduce anxiety in female rats [6f]. This is one possible explanation for the relaxing effect of the rose oil. Although our findings agree with other reports, it is important in future studies to investigate the active components that contribute to the deactivating effect on the sympathetic activity.

Correlation analysis between the ANS and behavior parameters showed that the decreases of systolic blood pressure, breathing rate, and blood oxygen saturation were not correlated with changes in behavioral responses (data not shown). These findings suggest the effectiveness of pharmacological mechanisms, for example 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 and then affect the $GABA_A$ receptor-mediated response, which would have a tranquilizing effect on the brain following inhalation or massage [7a,7b]. Therefore, one possibility that explains the relaxing effect of rose oil could be that the oil possibly stimulates the raphe nuclei in the brain into releasing serotonin, a neurotransmitter that creates a relaxing/sedative effect [7c,7d]. The raphe nuclei are also involved in sleep and relaxation [5b,7e]. Another possibility that could explain its effect is that rose essential oil interacts with central (e.g. hypothalamic, limbic, thalamus) structures which control the level of autonomic and/or behavioral arousal. All our findings indicate that differential effects of the essential oils depend on mode/route of administration. Both pharmacological and psychological effects are active simultaneously when the oils are administered by means of inhalation. In contrast, percutaneous administration provides evidence for pure pharmacological effects and the exclusion of olfactory processing. Therefore, in order to differentiate between pharmacological and psychological effects of fragrances, subjective evaluation of the odors must be prevented [6c,6d,8]. In conclusion, our investigation demonstrates the relaxing effects of rose oil and provides evidence for its use in medicines for the relief of depression and stress in humans.

Experimental

Subjects and essential oil: Forty healthy volunteers aged between 18 and 21 years (mean age 19.35 ±0.80 years) took part in the experiments. Demographic data for the control group and the experimental group are presented in Table 3. Subjects were tested in individual sessions and randomly assigned to either the control group or the rose oil group according to random numbers. They were fully briefed, given written informed consent to all aspects of the study (Srinakharinwirot University Ethics Committees) and were free to withdraw at any time. Forty-eight hours prior to testing subjects were asked to abstain from food, beverages and toiletries containing the essential oil as well as from any stimulants (e.g. caffeine and nicotine).

Table 3: Demographic data for the control group and the experimental group.

Parameter		Control group	Rose oil group
Number of volunteers		20	20
Sex (M:F)		10:10	10:10
Height (cm) (mean±SD)	Male	172.90±7.20	173.50±6.43
	Female	158.70±3.92	163.80±5.87
Weight (kg) (mean±SD)	Male	65.60±14.83	65.60±10.20
	Female	55.70±4.60	52.55±5.92

Rose oil was obtained by steam distillation of fresh petals of *Rosa damascena* (available from Thai-China Flavours and Fragrances Industry Co., Ltd., Thailand). The oil was analyzed by GC/FID and GC/MS. The oil contained citronellol (51.8%), geraniol (12.8%) and citronellyl acetate (5.2%). The minor components were methyleugenol (2.5%), caryophyllene oxide (1.6%) and farnesol (1.8%).

Essential oil administration: In the experimental group, 1 mL of a 20% (w/w) solution of rose oil in sweet almond oil was applied to the skin of the lower abdomen of each subject and the subjects self-massaged the oil into the skin for 5 min. Afterwards, the massage area was covered with a plastic film in order to prevent evaporation of the oil. In the control group, 1 mL of the placebo oil, pure sweet almond oil, was used. In both groups, subjects were supplied with pure air through breathing masks (inhalation set

for adult, product no.1500004020, B+P Beatmungsprodukte GmbH, Neunkirchen, Germany) in order to eliminate any olfactory stimulation by nose or mouth.

Experimental protocol: The experimental protocol has been previously described [6c,6d,8]. Briefly, one session consisted of two trials of 20 min each. At the beginning and at the end of each trial, emotional responses were assessed by visual analogue scales (VAS). Autonomic parameters were recorded continuously during 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 autonomic parameters: Blood oxygen saturation (BOS), breathing rate (BR), pulse rate (PR) and skin temperature (ST) 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 (Biopac Systems, Inc., Santa Barbara, California, USA). BR was measured using a SKT100C amplifier and TSD102D surface temperature thermistor probe. BOS and PR were assessed using a Pulse Oximeter Module (OXY100C) and a photoelectric transducer (TSD123B). ST was measured with a SKT100C amplifier and a fast response thermistor (TSD102A). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured in the dominant arm by sphygmomanometry using an automated system (Digital Electronic Model DS-155E, Japan). Details of the recording system and procedure have been described elsewhere [6c,6d,8].

Acquisition of 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: relaxed and tense for the item 'relaxation', vigorous and feeble for the item 'vigor', calm and restless for the item 'calmness', attentive and inattentive for the item 'attentiveness', cheerful and bad tempered for the item 'mood', alert and tired for the item 'alertness'.

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, e.g. sex, age, height, weight. In addition, they were asked about the rating of emotional responses. After completion of the interview and the rating scales, systolic and diastolic blood pressure (SBP and DBP) were measured. Subsequently, subjects were seated in a semi-reclined position, providing easy access to attach the ANS electrodes or probes. Electrodes were placed on suitable positions, as mentioned above. The breathing mask was fitted to the volunteer's face to cover the nose and mouth. Oxygen was then supplied directly. The oil or the placebo was administered, as described, together with recording of the autonomic parameters. After completion of the first trial, the subjects were asked to rate the VAS. The systolic and diastolic blood pressures (SBP and DBP) were measured at the end of the each trial. At the end of each trial, the subjects were asked if they had perceived any odor during the experiment. All subjects stated that they had not perceived any odor during the experiment.

Data and statistical analyses: The autonomic recordings of each subject were computed by trial using Acq*knowledge*® software (Biopac Systems, Inc., Santa Barbara, California, USA). For each subject and 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. For emotional ratings, on each scale the distance of the mark from the left-hand side was measured in mm. Individual difference scores between ratings were calculated for each item. The Statistical Package for the Social Sciences (SPSS version 11.5) was used for statistical analysis. Mann-Whitney-*U*-Test analysis of variances was used in this study. The effects of fragrances on autonomic parameters and ratings of emotional responses were determined by comparing the difference scores between the control group and the experimental groups. Correlational analyses between ratings of emotional responses and autonomic parameters were performed by means of Spearman rank-order correlation coefficient.

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