Aromatherapy: Evidence for Sedative Effects of the Essential Oil of Lavender after Inhalation

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Decrease of Caffeine, Hyperactivity, Lavender Oil, Linalool, Linalyl Acetate, Sedative Effects

The sedative properties of the essential oil of Lavender (*Lavandula angustifolia* Miller) and of its main constituents – linalool and linalyl acetate – were investigated in mice followed up in a series of experimental procedures. The significant decrease in the motility of female and male laboratory animals under standardized experimental conditions is found to be closely dependent on the exposure time to the drugs. Nevertheless after an injection of caffeine into mice a hyperactivity was observed which was reduced to nearly a normal motility only by inhalation of these fragrance drugs. In particular the correlation of the motility of the animals to linalool in serum is experimentally proven, thus furnishing evidence of the aromatherapeutical use of herbal pillows employed in folk medicine since ancient times in order to facilitate falling asleep or to minimize stressful situations of man.

Introduction

In folk medicine the use of lavender flowers (*Lavandula angustifolia* Miller) has been known for a long time [1–4]. Lavender flowers were filled in small linen bags and placed under the pillow mainly to prevent problems of falling asleep. This popular "therapeutic treatment" of mostly psychosomatic disorders, disregulations or indispositions of man only by inhalation of biologically active fragrant molecules, is called "Aromatherapy" or "Osmotherapy" [2, 5–7]. As already shown for peroral administration, a distinct depression of CNS activity was found for the essential oil of lavender and its main constituents [8–12]. Anticonvulsive effects, inhibition of the spontaneous motor activity and potentiation of the narcotic effect of chloral hydrate after peroral administration were shown in particular [8, 9] as well as specific sedative and spasmolytic effects on mice [10–12]. Until now no evidence was found that the essential oil of lavender can induce the effects as mentioned above only be means of inhalation. Still now, no scientific proof exists for an effective use of herbal pillows in folk medicine. Therefore, our aim was to study the aromatherapeutical influence of the essential oil of lavender and to prove their efficacy in herbal pillows used in folk medicine since ancient times.

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Materials and Methods

Animals

Female and male 6–8 week and 6 month old outbred Swiss mice with mean body weights of 28.5 gram were housed in groups of 4 animals under standardized conventional conditions (room temperature 22 °C ± 2°, relative humidity 60% ± 10, light-dark-rhythm 12:12 h, air exchange 12–15 times per h) on a bedding of wood shavings in polycarbonate cages (Makrolon®, type II). Standardized pelleted food T 779 (Tagger, Graz, Austria) and drinking water were provided ad libitum.

Chemicals

The essential oil of lavender (*Lavandula angustifolia* Miller, "Mont Blanc" quality) and its main constituents linalool (37.3%) and linalyl acetate (41.6%) were supplied by Dragoco-Vienna. To calculate the air concentration of the fragrance

compounds (Fig. 1) in the cage to consider the total drug volume we used the charcoal tubes LOT 120 NIOSH (Catalogue No. 226-01) supplied by SKC Inc., Pennsylvania 15330, U.S.A. Blood samples of 0.4 ml were collected from the mice by puncture of the retrobulbar venous plexus and each sample was mixed with 0.050 ml heparin (5000 I.U./ml, Immuno Inc., Austria). Caffeine was used as a 0.1% solution (1 mg/ml phosphate buffered saline) and injected intraperitoneally in volumes of 0.5 ml per animal.

1: R = H

2: R = $COCH_3$

3

Fig. 1. Formula of linalool (**1**), linalyl acetate (**2**) and 1,8-cineole (**3**).

Apparatus

In general, the experimental techniques have already been described elsewhere [13]. The inner measurements of the light-barrier cage were 41 × 24 × 8.5 cm and therefore resulted in a total air volume of about 8.4 litres. Two cages were used for each experiment and constantly filled with 150 ml of wood shavings as bedding and 12 pellets of food. The light-barrier, 2 cm above the cage-floor, was interrupted due to the motor activity of the animals crossing it and triggered impulses which were evaluated during the experiment. The mean air concentration of the essential oil resp. its main constituents in the cage was determined by means of capillary GC and GC/MS as follows: The air stream carrying the fragrance compounds was passed through a layer of activated charcoal which afterwards was eluted with carbon disulfide. After evaporation of the solvent the amount of fragrance compounds remaining in the air was determined by measuring the difference between the original amount of fragrance material in the glass tube and the residual amount in the charcoal. Thus 33 mg of lavender oil, 27 mg of linalool and 23 mg of linalyl acetate had to be in the cage air. A steady concentration by constant drug evaporation from glass tubes throughout the experiment was ensured.

Experimental procedures: In a series of previous experiments the time between 10 a.m. and 2 p.m. was found to be the highest motor activity period of the mice. Therefore the experimental procedures were started at noon. Two experimental cages with 4 animals in each were simultaneously used for one experiment, one group inhaled the investigated fragrant, the second group of untreated animals served as control. For the exposure of the animals to the fragrance compounds a small glass tube with a slit measuring 3 mm in width and 5 cm in length was used. The fragrance compounds were injected through a small hole of the cage wall and the rubber plug of the glass tube. Immediately after placing the mice into the cages and the horizontal fixation of the glass tube a transparent plastic seal (laboratory film, American Nat. Cam/Greenwich CT 06830) was fixed at the cage to form an airtight seal. A pumping-evaporating-system as a part of a spirometer system as described by Kovar *et al.* [13] was used to supply fresh air and to guarantee a steady air flow. One hour adaptation period was offered to the animals in which no pharmacological treatment occurred. The small glass tube was then filled with 1.5 ml of the respective fragrance material which was constantly released by the slit. The motor activity of the animals was measured during the 60 min adaptation time without treatment and 30, 60 and 90 min after filling the glass tube. Additionally 400 µl blood was taken after 0, 30, 60 and 90 min of the exposure time by puncturing the retrobulbar venous plexus. The blood samples were collected in heparin containing plastic tubes, plasma was separated by centrifugation, frozen and stored at −20 °C until use. Tiglinic acid benzylester as internal standard was added to the serum in a concentration of 10 ng/ml.

GC and GC/MS

A GC-FID from Carlo Erba (HRGC Mega Series) and a 50 m Carbowax fused silica column 20 M (HP) and a 25 m HP-5 column and hydrogene as carrier gas were used. For the GC/MS measurements we used a GC-MS equipment from Hewlett-Packard (5890 GC and 5970 MSD), the same colums and helium as carrier gas. The mass spectra have been recorded by means of an electron impact study (EI, 70 eV; detection limit:

1.0 ng/µl linalool in full spectra and 1.0 pg/µl in selected ion monitoring: SIM) within the range of 40–300 amu.

Statistics

Statistics were calculated using an Atari 1040 personal computer ("WISTAT" scientific statistic-package program). The significance was determined by Student's "t"-test and F-test, the level of significance chosen for p to reject the null hypothesis was < 0.05.

Results

The normal motor activity rates were found identical for female and male animals. Usually, untreated mice show a high tendency to explore their environment and to perform activities for social and physiological reasons (grooming, food and water uptake, etc). Under experimental conditions the sedative effects of the fragrants were expressed by the characteristic crouching of the animals in a corner of the cage. Rarely, single mice left the group to sniff at the glass tube containing the fragrant substances or to look for food and water. The inactive and drowsy behaviour of the treated animals was expressed as a significant decrease in the impulse counts.

In Fig. 2 a distinct suppressive effect on the motor activity of 6–8 week and 6 month old mice is shown. The motor activity of untreated control animals was arbitrarily fixed at 100%. A clear decrease of the motility of young mice after 30, 60 and 90 min was found after inhalation of essential oil of lavender, linalool and linalyl acetate (Fig. 2). Due to the weaker response on the motor activity of 6 month old mice a higher dose (3.0 ml) of the fragrance drugs was used. Thereupon, a significant motility reduction was induced by inhalation of lavender oil and its constituent linalyl acetate (data not shown).

In further experiments the mice were injected with caffeine and the motor activity was increased to a value of nearly 160% compared to the 100% of the untreated control animals. As a consequence of the administration of the fragrance drugs a distinct decrease of this hyperactivity was found (Table I).

Plasma levels after 30, 60 and 90 min of linalool are shown in Fig. 3 by GC-measurements. After

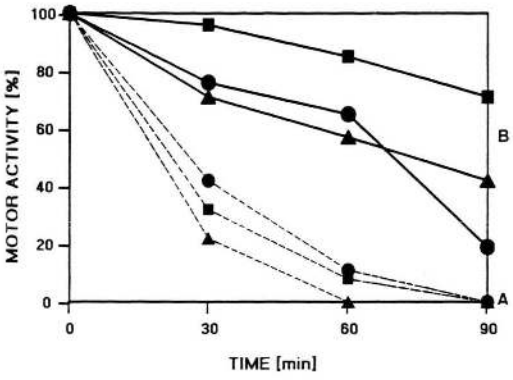

Fig. 2. Decrease of motor activity of 6–8 week old mice (A) and 6 month old mice (B) after inhalation of lavender oil (▲), linalool (■) and linalyl acetate (●). Motor activity values of untreated control animals was arbitrarily fixed as 100%. The motor activity was found 30 min after inhalative exposure to lavender oil: 22%/71%, linalool: 32%/96%, linalyl acetate: 42%/76%. 60 min after exposure to lavender oil: 0%/57%, linalool: 8%/85%, linalyl acetate: 11%/65%. 90 min after exposure to lavender oil: 0%/42%, linalool: 0%/71%, linalyl acetate: 0%/19%. The sample volume of each fragrance compound: 1.5 ml.

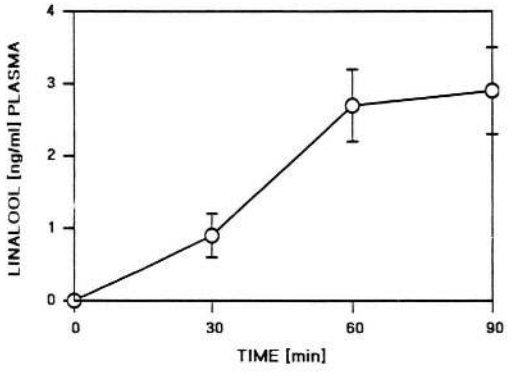

Fig. 3. The plasma levels of linalool in mice after the inhalation of this monoterpenic alcohol. (Means: ± S.E.M.; $n = 4$).

Table I. Additional activity after caffeine injection and exposure of the animals to the fragrance compounds 1 h after the administration of the excitant.

Caffeine:	+ 60%
Caffeine + lavender oil:	+ 5%
Caffein + linalool:	+ 26%
Caffein + linalyl acetate:	+ 32%

inhalation of lavender oil three signals *(m/z)* of linalool were differentiated in a GC/MS spectrum by 71, 93 and 121 amu in plasma samples of mice. The plasma content of linalool was determined using the benzyl ester of tiglinic acid as an internal standard. A direct correlation was found between the plasma concentration of linalool and the inhalation time.

Discussion

This study presents the evidence for a sedative activity of the essential oil of lavender and its main constituents, linalool and linalyl acetate after inhalative absorption. It is known that monoterpenic alcohols can have a spasmolytic, sedative and tranquilizing effect upon laboratory animals, such as mice, rats or goldfish [8, 10–12], which was frequently proved by means of oral or parenteral administration but never after inhalation*. In contrast to the oral administration in which dosages between 10 mg and 1000 mg per animal were used [11], a distinct effect after inhalative exposure was already found in the plasma of treated mice within the range of a few ng/ml as shown in Fig. 3 and as discussed by Kovar *et al.* [13]. Table II [15] summarizes acute toxicity studies of lavender oil and some terpenic compounds. These results lead to the conclusion that the very low concentrations used in the field of aromatherapy seem to be without pathological consequences on organs and tissue structures. Nevertheless, the aromatherapeutically used fragrance compounds are effective in causing and keeping the status of a sleep-like condition and sedation.

After inhalation the effectiveness of the essential oil of lavender and of linalool and linalyl acetate (see Fig. 2) differs between 6–8 week old and 6 month old animals. The reason for the higher threshold of effectiveness of the fragrance compounds in 6 month old mice can be explained by the higher amount of fat tissue in older animals which accumulates the very lipophilic terpenoid aroma compounds and reduces the effective plasma concentration. Also Teuscher *et al.* [16] stressed the lipophilic nature of the essential oils and consequently their membrane activity.

The gradually different decrease of the motor activity by linalool and linalyl acetate and the essential oil itself can be explained in two ways. A synergistic effect of some components of the essential oil is assumed leading to the improved effectiveness of the total substance itself. The main constituent of the essential oil of *Lavandula dentata* L., the bicyclic ether 1,8-cineole for instance, also a component of the essential oil of this study, is responsible for the distinct spasmolytic activity in calcium chloride dependent contractions [17]. It should also be noted that a stimulating effect of

* Kovar *et al.* [13] investigated the essential oil of rosemary and the ether 1,8-cineole in inhalation experiments. Römmelt *et al.* [14] studied the pharmacokinetic of essential oils and of some constituents of pine oils after inhalation with a terpene containing ointment.

Table II. Toxicity of lavender oils and some terpenic compounds.

Substance	Acute oral toxicity; rat LD_{50} g/kg	Acute dermal toxicity; rabbit LD_{50} g/kg	Percutaneous resorption	Skin-irritation
lavandin oil	5	5		neg.
lavender abs.	4.25	5		neg.
lavender oil	5	5		neg.
spike lavender oil	3.8	2		neg.
linalool	2.8	5.6	+	neg.
lavandulyl acetate	5	5		neg.
linalyl propionate	5	5		neg.
ocimene	5	5		neg.
α-terpinene	1.68	–		neg.
terpinolene	4.38 ml	5		neg.
1,8-cineol	2.48	5		neg.
camphene	5	2.5	+	neg.
bisabolene	5	5		neg.
caryophyllene	5	5		pos.

the essential oil of lavender was described by Schilcher in 1984 [18]. For this contrary effect the content of 1,8-cineole was found responsible acting as a motor stimulant [13]. Therefore, lavender oils with a high content of this bicyclic ether may cause a certain stimulatory effect which is highly dependent on the serum concentration of 1,8-cineole. Nevertheless, the essential oil of lavender mainly acts as a sedative drug.

The lesser decrease of motor activity in mice by the single components can be explained by their fate in the animals. Physiological metabolic activity of esterase hydrolyses linalyl acetate within a short period and causes the lack of a reasonable and effective concentration in the brain tissue. A similar metabolic pathway is also known for linalool which is metabolized as a primary alcohol to the water soluble glucuronide and eliminated by urine. By the way, this compound is unable to overcome the blood-brain barrier.

A striking observation due to the aromatherapeutical effect of this essential oil was made on the behaviour of hyperagitated, excited or stressed animals (Table I). After the intraperitoneal administration of caffeine the motor activity increased to a total value of nearly 160% compared to the activity of untreated animals. A significant decline of activity was achieved by inhalation of the fragrant materials one hour after caffeine injection. Two different declines were obtained: On the one hand after inhalation of the fragrant materials immediately after caffeine injection and on the other hand one hour after caffeine treatment. The reduction of the motor activity was significantly higher with regard to the elimination of caffeine by metabolic activity.

In contrast to the high doses of the described peroral treatment [11] the lower but effective doses of linalool incorporated only by inhalation are remarkable. The sedative action can either be explained by an excellent absorption by the nasal mucosa, leading to a serum content comparable to an intravenous injection within a short time (see [19]) or by pulmonary absorption [20] which was investigated for pine needle oil, rosmary oil and hay-blossom bath oil. The observed sedative effects of these fragrant substances were caused by a pharmacological efficacy on the brain and not by means of any reflected influence caused by comfortable aromatic effects on the olfactoric sense. In folk medicine the status of a pleasant feeling after inhalation of a delightful fragrance was suggested, *i.e.* pacifying a stressfull and tense situation. But it is known [16] that such lipophilic substances as essential oils interact with membrane lipids of the cells, thus causing by means of a cascade of reactions narcotic effects.

Although extensive pharmacokinetic studies are still lacking, biological effects on the motor activity of young and older mice were shown. The direct action of the fragrance molecules upon the central nervous system of the animals caused distinct sedation. Similarly direct pharmacological effects were also found on stimulating motor activity with 1,8-cineole [13]. These findings are in agreement with the reports of Römmelt *et al.* [20] indicating that most of the terpenes were distributed in several tissue compartments.

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