Methods in Aromatherapy Research

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The use of essential oils and single volatile chemicals for healing purposes has been known in folk medicine since ancient times and is discussed nowadays because of the trend back to natural drugs and therapies in medicine.[1] The term aromatherapy was coined in the late 1920s by the French cosmetic chemist R.M. Gattefossé, who noticed the excellent antiseptic properties and skin permeability of essential oils. He defined aromatherapy as any therapy with essential oils.[2] Anticipating the trends of the 1980s, "New Age" and esoterics, R. Tisserand revived this term by including it in a general natural healing method with elements of wholistic, cosmic, anthroposophic and other phenomena.[3]

Nowadays many of the so-called aromatherapists, devoid of any serious scientific training, use this definition for their own purposes, combining it with mystic elements[4] and uttering such nonsense as "essential oils are the soul, the spirit, the personality of the plant."[5] All this leads to confusion or misinterpretation. The word *aromatherapy* is a combination of aroma, which means "fragrance, sweet smell, a subtle quality,"[7] and therapy, which implies a handling by doctors or persons trained in medical sciences. But many aromatherapists deny the close correlation of aroma to volatiles, although volatility is the distinguishing characteristic of essential oils!

We need a universal definition with a scientific basis. Therefore, I have proposed the following definition of the word *aromatherapy: Therapeutic uses of fragrances or at least mere volatiles to cure and to mitigate or prevent diseases, infections and indispositions only by means of inhalation.*[8,9]

Following this definition, serious scientific work on the biological effects of volatiles, especially essential oils, was compiled with the aim to put a clear line of demarcation between a scientifically based aromatherapy and such aromatherapy as is widely practiced today. This paper summarizes and discusses the methods that have been applied to establish clear scientific facts of the efficacy of inhaled volatile chemicals.

The methods to establish proofs of the physiological, non-psychological, and pharmacological activities of volatile chemicals can be divided in two groups: animal experiments and experiments with human volunteers.

Animal Experiments

Tests with various animals by administering volatile chemicals either perorally or parenterally to check their toxicity and their pharmacological behavior are as common as skin trials to assay their allergenic potency or healing properties. But such experiments are not the topic of this review, because the main point in aromatherapy is the inhalative uptake of the fragrance molecules and their resorption by the nasal and/or pulmonal mucosa. In this respect, only two groups of scientists are known in literature up to now to have studied the behavior of test animals under the influence of volatile compounds. The earliest work was done by Kovar and Ammon et al. in Tübingen, Germany. It was followed by the work of my team in Vienna. The method of choice was to examine the motor activity of test animals, preferably mice, in a cage with a light barrier.

Kovar on mouse motility: Kovar and Ammon et al. showed interest in establishing an unambiguous proof of the efficacy of the essential oil of rosemary,[10] used in phytotherapy as an activating, refreshing remedy for exhaustion. It could be shown that by means of the main constituent of this essential oil, namely the bicyclic ether 1,8-cineole, this phytotherapeutical treatment is justified. The locomotor activity of the test animals increased significantly by inhalation of this material. More important, these scientists succeeded in showing a clear pharmacokinetic dependence on 1,8-cineole: The higher the concentration of this fragrance in the blood of the mice, the higher also is the rate of interruptions of the light barrier, indicating an increased motor activity of the test animals. Ammon later showed by similar studies that the essential oils of balm leaves and valerian roots have a calming and relaxing effect, whereas the essential oils of dwarf pine needles and juniper berries have the same activating effect as the oil of rosemary.[11]

Buchbauer on mouse motility: The Buchbauer team studied the effects of single aroma chemicals and essential oils inhaled by test animals (mice). In accordance with the relevant literature,[12,13] volatile essential oils were chosen and tested in an animal model under standardized conditions.[10,14,15] To date, the Buchbauer team has investigated 44 chemicals and essential oils with ascribed sedative effects on humans. These materials were used in inhalation experiments on untreated and pretreated mice. The pretreated mice were over-agitated by an intraperitoneal dosage of caffeine. The studies showed that lavender oil, its main constituents linalool and linalyl acetate, as well as neroli oil, citronellal, 2-phenylethyl acetate, α-terpineol, benzaldehyde and East Indian sandalwood oil, decreased

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the motility of untreated mice by 40–78%, compared with the control. Tests with pretreated mice also showed a significant decrease in activity; lavender oil, linalool, isoeugenol, maltol, methyl salicylate, carvone and linalyl acetate produced a 46–91% reduction in activity. In other investigations, blood samples obtained from mice having inhaled such volatile chemicals were analyzed by means of GC-spectroscopic systems to detect and identify possible active compounds responsible for the motility decrease.[10] In some cases volatiles could be identified in the blood in the range of 0.12-10.7 ppb (benzaldehyde after inhalation).[1]

Finally it should be pointed out that aroma chemicals really are resorbed and are able to cross the blood-brain barrier. After a 15-minute inhalation of a certain aroma chemical, mice were examined. The chemical could be detected in the blood and in the cortex, but not in the medulla.[16]

Experiments with Human Volunteers

We need to set a clear line of demarcation between aromatherapy, where physiological and pharmacological activities of fragrances are concerned, and aromachology, where the interrelationships between psychology and odors are discussed.[17] Only those research methods which offer a better insight into the complex reaction of the human body during inhalation are reviewed in this paper, to be consistent with the new definition of aromatherapy given above. Consider what happens in the human body during inhalation. In response to the chemical stimulus, there is what I call a "reflectoric" event, which is an event caused by a reflex. For example, if you smell and/or see something appetizing, you produce more saliva in your mouth and more fluids in your stomach. These are reflectoric events. There is also a biological event as molecules are resorbed. Naturally it is very difficult to separate these two events. But it is very important to separate them because one event deals with the effects that are achieved through stimulation of the olfactory pathways in the brain, and that's the field of aromachology.[17] The other event deals with reactions which are obtained by introduction of the fragrance molecule into the blood stream by nasal and/or pulmonal resorption, and that's the field of aromatherapy.

Regarding the methods which have been applied to put more light on the question of the body response upon an inhalative uptake of fragrance molecules, it is interesting to notice that Japanese scientists are at the forefront of aromatherapy research. An excellent review on the effects of odors, published in 1990 by Kawasaki,[18] makes it easier for this author to compile the research work done up to now on aromatherapy.

SPL: Torii investigated the effects of odors upon skin potential levels (SPL).[19] SPL are related to mental perspiration and correspond well with the arousal level of the subject: –40mV upon awakening, –60mV at times of excitement and near 0mV during sleep. Regarding psychophysiological effects, Torii reported that the scent of chamomile oil produced a sedative effect, while the scent of jasmine oil was stimulating. Measurements of the SPL via the contingent negative variation (CNV) clearly showed the variation of the SPL in parallel with the level of activity of the sympathetic nervous system. According to Niimi,[20] such tests indicated that pleasant as well as unpleasant odors raise arousal levels, and that especially the latter cause relatively large potential fluctuations.

CNV: The above-mentioned contingent negative variation (CNV) is a slow upward shift in brain waves recorded by the electroencephalogram (EEG), occurring in situations where the subject is attentive, expecting that something will happen. The first to use this method—known in brain research since Walter et al.[21]—were Torii and his team, who studied the CNV and the physiological effects of odors in humans.[22] The results obtained so far show that jasmine oil brings about an effect similar to the administration of caffeine, namely an increase in CNV after inhalation, whereas lavender oil causes CNV to decrease, similar to the administration of tranquilizers, thus leading to sedation. Other essential oils were also studied, and their effects upon inhalation were documented.

Recently Kubota et al.[23] reported on similar investigations using essential oils, several fractions obtained by vacuum distillation, and even single aroma chemicals. In some cases, they were able to show distinct differences between chiral molecules in their ability to influence the CNV. For example, l-carvone elicited a sedative activity, whereas its caraway-like-smelling antipode d-carvone acted as a stimulant.

Peripheral blood pressure: Measurements of fragrance effects by plethysmography have been performed by Konishi et al.[24] This is a method which observes the changes in the peripheral blood stream caused by the sympathetic part of the autonomic nervous system. Stress situations cause a constriction of the peripheral vasculature, hence increasing the peripheral blood pressure. Inhalation of the odors of the essential oils of peppermint and jasmine produced neurophysiological activities which led to a loosening of an induced stress situation, and subsequent relaxation of the maximum constriction of the peripheral vasculature. Of the two fragrances, jasmine odor had a slightly greater effect in reducing peripheral blood pressure.

Heart rate: Another physiological event can be used to observe the effects of odors: the heart rate. Oguri et al.[25] showed that pleasant odors stimulating the central nervous system make the heart rate more variable, whereas sedative odors decrease heart rate variability. Along the same line, experiments by Kikuchi et al.[26] showed that rose oil suppressed the deceleration of the heart rate, indicating activation, whereas lemon oil enhanced it. Thus the pattern of changes in the heart rate appears to show differences between stimulant odors and sedative ones.

Electrodermal activity: Two other methods to examine the effects of odors on humans have been applied by the German fragrance chemist W. Steiner. In one method, Steiner measured electrodermal activity, which is changes in the steady current between two points at the skin surface. Differences in electrodermal activity between twelve test odors enabled the scientist to characterize the odors as either sedatives or activators.[27]

Pupil dilation: Another method used by Steiner measures the dilation or constriction of the pupils.[28] Results showed that all odors under investigation caused a pupil enlargement, indicating arousal. Miyazaki et al.[29] used Steiner's procedure in a cognitive task and found that orange oil increased the parasympathetic nervous system activity (indicated by a distinct acceleration of the constriction rate of the pupil), whereas the sympathetic nervous system activity was found to be decreased.

Brain waves: We have already discussed the CNV method for measuring electrical activity in the brain, and its use in aromatherapy research. Other methods to investigate the influence of volatile materials on brain waves should be mentioned. In particular, the teams of Lorig at Washington, van Toller at Warwick (UK) and Kobal at Erlangen (Germany) are famous for their pioneer work on the effects of odors on mood or feelings.[30-35] But these investigations are more in the field of aromachology than aromatherapy, especially when hedonic qualities exert an influence on the results.

Nevertheless, Japanese scientists again showed that evoked potentials and brain wave studies can be used in aromatherapy research. Steady-state waves can be distinguished by their frequency: α-waves are characterized by a frequency of 8-13 Hz, while those with higher frequencies are called β-waves and those with lower frequencies ϕ- and δ-waves. β-Waves are dominant when a subject's brain is engaged in concentrated thought or the subject is in a highly emotional mental state. An α-wave dominancy is characterized by a mentally relaxed state or a subject in meditation. By examining the EEGs of volunteers exposed to various essential oil odors, Sugano found that the odor of either lavender oil or sandalwood oil increases α-wave activity, whereas jasmine oil odor increases β-wave activity.[36,37] Finally, Lorig and Schwartz reported that spiced apple aroma relieves stress and promotes a sense of relaxation, as demonstrated by a reduction of the higher frequency activity and an increase in the lower frequency activity in EEGs.[38]

Cerebral blood flow: Sugano also reported on a totally new method to "look into" the brain.[37,39] Using the fragrances of lavender, jasmine, sandalwood and α-pinene as olfactory stimuli, Sugano used Xe-positron-emission tomography to measure the local cerebral blood flow (CBF) in order to observe the events in the brain upon inhalation of these fragrances. He compared his findings with the results he obtained by means of measurements of the α-brain wave activity as well as with results obtained by CNV experiments. He found a great similarity among all these data achieved by different methods: α-pinene, sandalwood and lavender elicited a distinct sedation, whereas jasmin exerted a stimulating effect.[37,39]

Two new methods in aromatherapy research introduced by my own team should be discussed here. As mentioned earlier, Ammon stated that 1,8-cineole must affect the motor centers in the brain.[11] Ch. and B. Nasel proved this result by performing Xe-computer-tomographic studies on healthy volunteers with the view to observe the cerebral blood flow upon inhalation of 1,8-cineole.[40] While Sugano used the positron generating radioactive 133Xe in his PET-method,[37] my Viennese group used stable, gaseous Xenon (not more than 30% in the normal breathing air) which serves as a contrast medium for computer-tomography measurements during the examination procedure. This is a three-dimensional, high-resolution method to study the regional cerebral blood flow (rCBF) for clinical routine. It allows direct analysis of the events in the blood supply within the investigated brain structures[41] and seems to become ever more irreplaceable in neurology.

Eight neurologically healthy volunteers between 20 and 30 years old, and one clinically tested anosmic person, were examined before and after inhaling 1,8-cineole. In all cases the CBF of these subjects increased when they inhaled this compound, showing an enhanced cortical activity. The anosmic woman reacted in exactly the same way. If, therefore, even an anosmic—a person who really is unable to create a pleasant feeling upon sniffing a fragrance—shows an increased CBF, it cannot be the result of a reflectoric event. In addition, the concentration of 1,8-cineole in the blood was determined. Resorption is very fast, as could be shown during the period from 4 to 20 minutes by an almost linear increase of the 1,8-cineole concentration up to a maximum value of about 275 ng/ml serum.[42] When the inhalation was stopped, the concentration of this chemical in venous blood dropped immediately.

With transcranial Doppler (TCD), a sonographical, noninvasive imaging of vessel flow velocity is possible. TCD is an ultrasound method based on the Doppler shift principle, which means a frequency shift of a sound signal when either the source or the receiver is moving. A movement of one towards the other is defined as a positive shift, while movement of one away from the other is a negative shift. In such TCD measurements, "speed" is expressed by flow velocity. An ultrasound signal is sent into the tissue and is reflected by the moving blood particles, mainly the erythrocytes.[43] This new method is the second innovation introduced by the Nasels, members of my team, into aromatherapy research. The results obtained upon examination of some healthy volunteers and the anosmic subject, again while inhaling 1,8-cineole, are nearly the same as with Xe-CT.[44]

Conclusion

Many methods have been applied in aromatherapy research in order to verify the so-called healing effects of aroma chemicals and essential oils. Even if it is very difficult to separate the psychological (desired healing effects from a reflectoric event) from the pharmacological (direct interaction of the fragrance molecules with the appropriate receptor systems), many of the aforementioned methods are used in aromachology research as well as in aromatherapy research. But, by applying new investigation methods on an anosmic person, I proved for the first time that essential oils and aroma chemicals—aside from creating a mostly happy feeling—do behave like pharmacologically active substances. For the first time it was possible to show that fragrance molecules really get resorbed, are transported in the blood stream and thereby elicit distinct effects in human subjects.

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