Unbiased research is needed for rational translation of essential oils in clinic

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Abstract. The use of complementary and integrative therapies is steadily growing though the quality of clinic evidence for the use of essential oils is hampered by several methodological biases. Lack of rigorous methodology in clinical studies with aromatherapy originates poor quality evidence and scientific response to overcome the biases of this field of research is needed. Accordingly, here we display a possible step-by-step preclinical-to-clinical pathway, that was followed for the essential oil of bergamot (BEO), to overcome typical biases of research in the field of essential oils, in order to provide good quality body of evidence.

Key words: bias, clinical aromatherapy, bergamot essential oil, NanoBEO, severe dementia, pain, agitation, I-MOBID-2, CONSORT.

Poor clinical research on natural products

The Food and Drug Administration (FDA) classifies essential oils for aromatherapy as cosmetic formulations. This is in line with the poor methodological rigor of clinic research in this field as well as in the field of nutraceuticals (1) and of neuroprotective agents in general (2), impeding to draw any definite conclusions about efficacy and safety of these interventions for clinical translation. In fact, in spite of the continuously growing market of natural products and the increasing use of integrative medicine, advances to provide high quality evidence for the translational and clinic use of these products is needed. The poor quality of preclinical research contributes to the biased clinical research (3). One of the fields in which aromatherapy is widely studied is that of behavioral disturbances, often during dementia. In fact, dementia has a remarkable global burden since 55 million people suffer from this neurodegenerative disease, getting even more important during the Coronavirus disease (COVID)-19 pandemic since it increases the risk of death of these patients (4). Dementia, of which Alzheimer's disease (AD) is the most common form, is fundamentally linked to the development of neuropsychiatric symptoms (NPS) in about 97% patients that worsen their health-related quality of life (HRQL) (5). NPS and agitation in particular are tightly associated to unrelieved pain that undergoes altered processing in aging (6). In fact, up to 80% nursing home elderly, that represent the population most

affected by AD, present age-related comorbidities associated with chronic pain as rheumatic conditions (7-9), low back pain (10), stroke (11), post-herpetic neuralgia and diabetic or chemotherapy-induced neuropathies (12). It is under diagnosed and under treated, also in the community setting (13-15), because cognitive deterioration impairs the self-report skills (16). Agitation is treated with atypical antipsychotics that are linked to increase up to almost double of risk of death for cardiocerebrovascular accidents (17). Due to the evident correlation between development of resistant agitation and unrelieved chronic pain and since pain severity is associated with NPS and with the use of antipsychotics (18), analgesic therapy has been tested resulting to provide effective management of agitation (19). Analgesia is the most efficacious treatment for the management of NPS (20) and it reduces the need for antipsychotics (21, 22), Therefore, aromatherapy and integrative medicine gains interest in this field, although no evidence for efficacy can be drawn, also according to Cochrane systematic review, for the methodological flaws of clinical trials (23, 24).

Biased studies

Conducting a search on PubMed/MEDLINE applying the filters to retrieve randomized clinical trials published in the last 5 years up to June 28th, 2022 with the query string "aromatherapy AND dementia" 7 results are obtained (25-31) (figure 1).

Within the studies retrieved several sources of bias occur. The study by Mascherona and collaborators enrolls 32 patients (n=16 for the control and n=16 for the intervention) to investigate the effects of aromatherapy by environmental diffusion complemented with standard psychotropic therapy and Pro Re Nata (PRN) in comparison with standard psychotropic therapy and PRN alone on NPS (29). Aromatherapy by environmental diffusion has the intrinsic bias of not allowing titration of the active components of the phytocomplex used and to prevent exact reproducibility because the concentration can be subjected to modifications also due to the different environmental conditions. Moreover, aromatherapy permits the identification by the patients and the operators of the intervention, since essential oils are endowed with strong

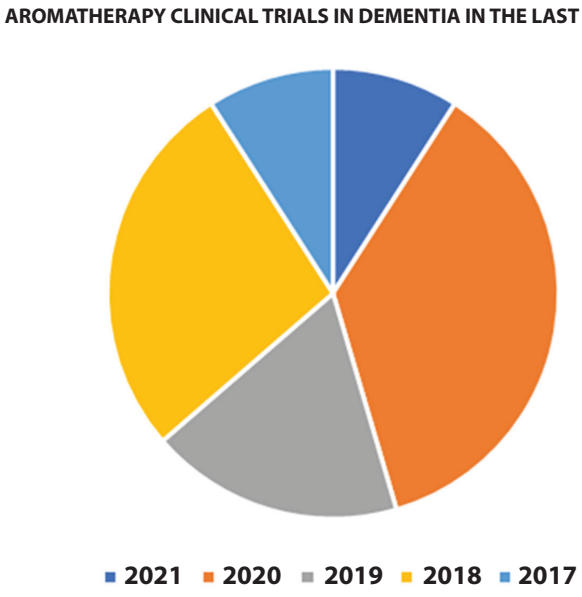

Figure 1. Randomized clinical trials retrieved from PubMed/MEDLINE in the last five years using the search string "aromatherapy AND dementia" (date of last search June 28th, 2022).

aroma, thus inducing concealment and reporting biases. Aromatherapy and massages are used also in the studies of Dimitriou and colleagues (25, 26) and in the study by Takahashi and collaborators (30) and the trial by Watson et al. (31) used a cotton patch attached the cloth to the participants collar area. The study by Fung et al., (27) uses a multicomponent aroma-massage and the study by Kozuki and coworkers uses aroma oil as a bath salt (28). Another source of bias is represented by the high possibility of essential oils to undergo degradation, causing the change of concentration of the active ingredients content. Furthermore, the samples do not always result from a rational calculation, and they are often small.

Alzheimer's disease (AD) and related agitation

Alzheimer's disease (AD) is the most common type of dementia, accounting for two thirds of total cases (32, 33). Among the several disturbances characterizing dementia, some of the most widely known are cognitive deficits and memory impairment, but 97%, thus almost the totality, of patients develops fluctuant neuropsychiatric symptoms (NPS), known as behavioral and psychological symptoms of dementia (BPSD), during the course of the disease and even before its onset (5). The latter disturbances, according to the International Psychogeriatric Association (IPA), are "a heterogeneous range of psychological reactions, psychiatric symptoms and behavior occurring in people with dementia of any etiology" (34, 35), often causing institutionalization (36). AD is a continuum characterized by an insidious onset and a median increase of the Neuropsychiatric Inventory (NPI) score at 5 years from diagnosis is reported (37); in particular, NPS can represent an under-recognized risk factors for AD development (38). Decreased motivation and affective dysregulation (39) characterize prodromal mild behavioral impairment (39, 40). People suffering from moderate-to-high depressive symptoms have been reported to be at increased risk to develop mild cognitive impairment (MCI) (41, 42). In fact, MBI represents the development of NPS in physiological aging, or in people with subjective concerns of cognitive decline (SCD) or suffering from MCI as at-risk state for incident cognitive decline and dementia (43). In particular, NPS in course of MCI are fluctuating and consist in apathy, depression, agitation, delusions, hallucinations, and sleep disorders that represent a higher risk of conversion to dementia (44). Depression in MCI doubles the risk to develop dementia (45). The severity of dementia has been correlated with hyperactivity, psychosis, affective symptoms and apathy (46). Moreover, the severity of cognitive decline is related to psychosis (47) and increase over time of agitation, disinhibition, irritability and aberrant motor behavior (48). Correlates of intracerebral pathology in course of mild, predementia symptoms can be highlighted by the progresses in structural and functional neuroimaging and in the biochemical analysis of cerebrospinal fluid occurred during the last decade (49). In fact, spatial patterns of neuroimaging biomarker change highlighted that amyloid beta (Aβ) increase significantly already 22 years before symptoms along with glucose metabolism decrease, as demonstrated in a study on rare genetic mutations (50). Also metabolomics highlighted the involvement of altered metabolism of branched-chain amino acids in AD (51). Agitation is one of the most challenging NPS and it can be induced by several triggers and as response to different situations of discomfort (52) including: depression (53), disturbance of the night-time sleep pattern (54, 55), constipation (56) and changes in environment, over or under stimulating (57). Moreover, the use of drugs as benzodiazepines in dementia deserves caution since they can exacerbate these symptoms (58, 59). Also, sensory impairment, acute medical illness (e.g., infections, respiratory diseases, urinary retention, renal failure and hospitalization), or metabolic changes, psychological distress, including delirium and depression, and the reduction of natural light in the evening in the case of sundowning syndrome, can induce agitation (60-63). Depression and anxiety are more frequent in younger patients; on the contrary, agitation, disinhibition, irritability, and aberrant motor behavior together with psychosis increase over time with the severity of dementia (48). Low socioeconomic status is a risk factor for the development of dementia and a population- and register-based cross-sectional study investigated the correlation of dementia diagnosis and cognitive stages at diagnosis (MCI, mild, moderate, or severe

dementia) with age group, sex, region of residence, household type and therapy (64). It shows that the socioeconomic status influences the referrals for diagnostic evaluation for dementia and these patients are often women, with lower educational level and multiple medical conditions (64).

Current treatments for AD-related agitation and the role of analgesia

Disease-modifying drugs are still lacking, in spite of the recent accelerated approval of aducanumab [35] by the Food and Drug Administration (FDA). Therefore, the current symptomatic anti-AD therapy against cognitive decline consists in acetylcholinesterase inhibitors and the low affinity non-competitive N-methyl-D-aspartate (NMDA)-receptor antagonist memantine, according to the Mini-Mental State Examination (MMSE) score. In the highest majority of cases, AD occurs in patients ≥ 65 years old, not being part of physiological aging (65). Therefore, affecting mainly the population of the elderly, it overlaps with age-related comorbidities responsible for chronic pain (66): musculoskeletal pain, including inflammatory arthritis, osteoarthritis and disorders related to soft tissues (67); diabetes with diabetic peripheral neuropathy and peridiabetic lesions (68) as neurological complications of diabetes; herpes zoster and post-herpetic neuralgia, being a common sequela of dermatomal rash in the older adult (69); advanced cancer and breakthrough pain (70). Aging can impact pain processing and a tight correlation between behavioral disorders, particularly agitation, and inadequate pain relief has been demonstrated (71-73). Indeed, analgesia is more effective than other treatments of agitation (20), that can be significantly reduced by means of appropriate pain treatment and regular review of therapy (21, 22). In particular, oral non-steroidal anti-inflammatory drugs (NSAIDs) including naproxen, ibuprofen and diclofenac are used for inflammatory musculoskeletal pain, while celecoxib for the treatment of chronic osteoarthrosis, after failure of acetaminophen, only for short periods as recommended by the American Geriatric Society (AGS) panel (74, 75), to reduce the gastrointestinal, renal and cardiovascular adverse reactions (76-78) and with caution in case of warfarin concurrent use (79). Gabapentin/pregabalin (80) are indicated for the treatment of neuropathic pain; serotonin-noradrenaline reuptake inhibitors (SNRIs, i.e. duloxetine, venlafaxine) (81) can be used, but not tricyclic antidepressants (TCAs, e.g. amitriptyline) due to their cardiovascular contraindications (82). Tramadol, tapentadol, buprenorphine or transdermal fentanyl after effective dose titration can be required for the treatment of severe chronic pain conditions (83, 84), following the paradigm "start low and go slow" (85), considering liver and/or renal failure. The pivotal role of chronic pain in the development of agitation is supported by the evidence that adherence to symptomatic treatment targeted towards cognitive decline can delay the onset, but not prevent the development of agitation (86). Up to 80% of patients suffering from dementia during their stay in long-term care facilities experiences pain (87). In particular, non-verbal, severely demented patients often receive insufficient pain treatment (19), due to impaired communication skills that make pain diagnosis and assessment more difficult than in cognitively intact peers (88). Moreover, the oldest old, mainly stroke survivors and cognitively impaired (11), generally are excluded from clinical trials (89), particularly for migraine (90-92). The only approved treatment for agitation is represented by the use (for no longer than 6-12 weeks) of the atypical antipsychotic risperidone (21, 93-95). Nevertheless, the use of neuroleptics in this fragile population is known to double the risk of death for cardiocerebrovascular accidents (17). An effective and safe therapy for agitation is not available yet. *Melissa officinalis* and *Lavandula officinalis*, two phytocomplexes in the form of essential oil, have proven some efficacy in the management of agitation (21). Despite this, the quality of the latter evidence is hampered because of methodological biases, as it is the case for all essential oils used in aromatherapy clinical trials, (23); accordingly, no definite conclusion about the efficacy of intervention with essential oils in dementia can be drawn (23, 96). In fact, already two decades ago (24) lack of adequate methodology in clinical studies was underlined, and in face of the increased number of trials investigating aromatherapy (figure 4) their level of certainty has not significantly improved and effort is still scarce in the study of pharmacokinetic interactions (97). A search for clinical trials has been conducted (date of last search May 17th, 2022) screening the database PubMed/MEDLINE for the following search queries: "aromatherapy", "essential

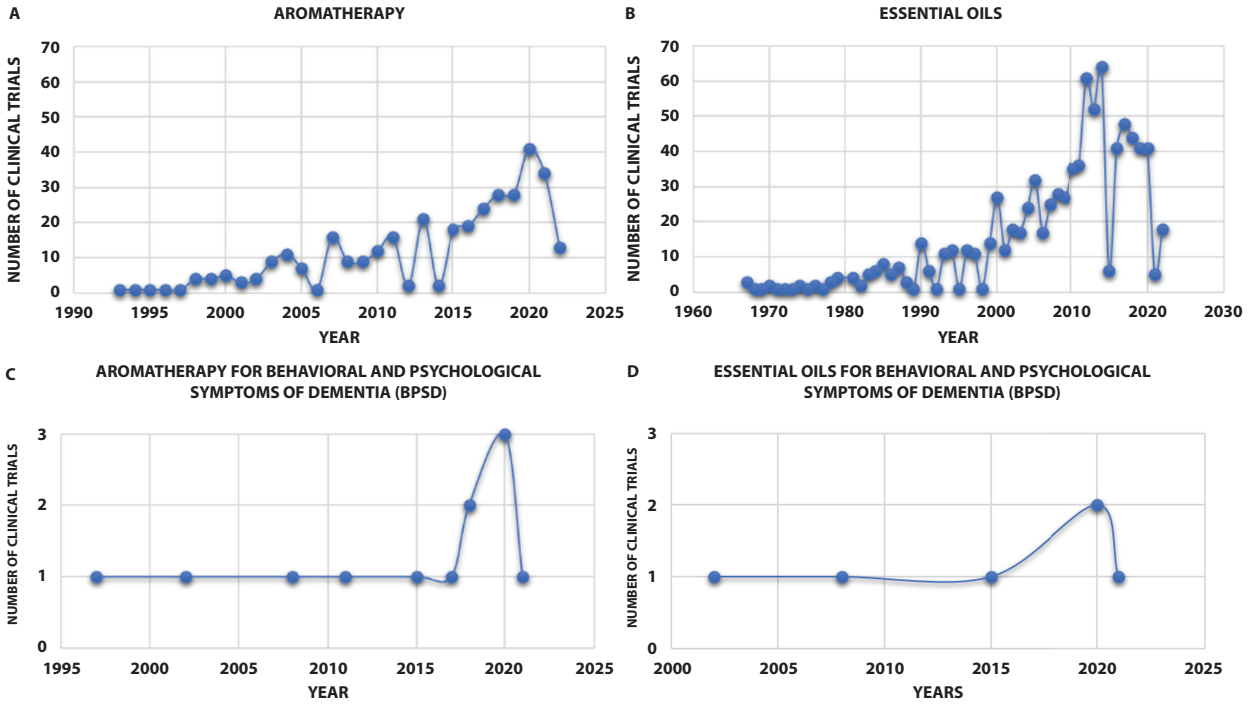

Figure 2. Clinical trials investigating aromatherapy/essential oils and behavioral and psychological symptoms of dementia since PubMed/MEDLINE inception (date of last search May 17th, 2022).

oils", "aromatherapy AND behavioral and psychological symptoms of dementia", "essential oils AND behavioral and psychological symptoms of dementia". According to the retrieved results, 345 clinical trials have investigated aromatherapy since 1993, up to a peak per year of 41 in 2020 (figure 2a), and only 12 of these where concerned with neuropsychiatric symptoms often called behavioral and psychological symptoms of dementia (BPSD) (figure 2b). The latter observation is strengthened when considering the finding that use of essential oils, are tested in 866 clinical trials from 1967 to present with a peak of 64 in 2014 (figure 2c), among which only 6 regard BPSD (figure 2d), supports the lack of improvement in this field.

Engineering of the essential oils allows to overcome the biases of aromatherapy clinical trials

Agitation in course of dementia can be due to unrelieved pain, hence it can be safely managed through analgesia. For the reasons discussed above, *Melissa officinalis* and *Lavandula officinalis* control of agitation is not conclusive and, more importantly, they do not show strong preclinical analgesic properties. Therefore, an essential oil proving antinociceptive action in experimental pain modelling clinic conditions can represent the best option for clinical translation into the management of AD-related agitation. Cannabinoids deserve attention in the field of pain, dementia and stroke (98, 99). The taxonomy, origin, biodiversity and phylogeny of the *Citrus* species is very complex, diversifying during the late *Miocene* epoch (100) and within this genus the essential oil of bergamot (BEO), classified as *Citrus bergamia*, Risso belonging to the *Rutacee* family, is a hybrid late in phylogeny likely originated in the southern part of Italy and, in particular, in Calabria (101, 102). In agreement with the *Farmacopea Ufficiale Italiana* it is obtained by cold pressing of the epicarp and, partly, of the mesocarp of the fresh fruit (103). The oxygenated compounds mainly responsible for its pharmacological activity are linalool, linalyl acetate and the terpene limonene (104) contained in the volatile fraction. BEO is the sole to have proven sound, rigorous, preclinical evidence of analgesic efficacy both in acute, inflammatory (105) and neuropathic

(106, 107) pain, but also in the formalin test relevant to clinic conditions due to its central sensitization mechanisms based on the criteria for critical appraisal of preclinic research (108, 109). Moreover, BEO is endowed with anxiolytic-like effects devoid of sedative action of diazepam and benzodiazepines in general (110), linked to the modulation of serotonergic mechanisms in the animal behavioural tasks Open Field Test, Elevated Plus Maze Test and Forced Swimming Test (111). The phytocomplex deprived of bergapten to avoid phototoxicity (112) is encapsulated in a nanotechnology delivery system based on solid lipid nanoparticles (SLN), NanoBEO (113). SLN are enriched with the anti-oxidant α-tocopherylstearate (α-TFS-SLN), to entrap the aroma, and they are incorporated into a cream for transdermal administration. This technological manipulation allows to maintain the antinociceptive and antiallodynic properties of BEO solving the issues of trials in aromatherapy to prove clinical efficacy and safety affording: 1) titration of the active principles; 2) increased stability to heat and light and consequent prevention of the degradation of the active components. This aspect is fundamental since the different active principles are responsible for the analgesic effects of BEO (114, 115); 3) reproducibility of effects thanks to constant concentration; 4) double-blind clinical trials are allowed because aroma is entrapped making NanoBEO and placebo cream indistinguishable. NanoBEO cream is dispensed through an airless dispenser preventing degradation and allowing feasibility of exact dosing (116). Furthermore, NanoBEO proves efficacy on scratching behavior that is a typical NPS. NanoBEO is patented (EP 4003294) and its efficacy and safety on agitation and pain in patients aged over 65 with severe AD is now under investigation in the first high-quality, registered (NCT04321889) (117) actually recruiting randomized, double-blind, placebo-controlled clinical trial adequately powered (n=134 patients are going to be enrolled) and following the Consolidated Standards of Reporting Trials (CONSORT) (118) statements. Since severe dementia impairs the self-report of pain the Italian version of the Mobilization–Observation–Behaviour–Intensity–Dementia (I-MOBID2) (119) recently validated in the Italian setting is going to be used to guarantee an accurate evaluation of musculoskeletal and visceral pain and to unravel even concealed pain conditions because of the execution of five guided movements (120, 121). The present step-by-step preclinical-to-clinical pathway can form the rational basis for a definite, effective and safe treatment of agitation treatment for the fragile population affected by severe AD.

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