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Review article

Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial

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Abstract

S. lavandulaefolia Vahl. (Spanish sage) extracts and constituents have demonstrated anticholinesterase, antioxidant, anti-inflammatory, oestrogenic and CNS depressant (sedative) effects all of which are currently relevant to the treatment of Alzheimer's disease (AD). The essential oil inhibits the enzyme acetylcholinesterase (AChE) from human brain tissue and bovine erythrocyte and individual monoterpenoid constituents inhibit AChE with varying degrees of potency. In vivo AChE inhibition of select brain (striatal and hippocampal over cortical) AChE was obtained following oral administration of the essential oil to rats. In a study in healthy volunteers essential oil administration produced significant effects on cognition. In a pilot open-label study involving oral administration of the essential oil to patients with AD, a significant increase in diastolic and systolic blood pressure was observed in two patients, however this may have been due primarily to preexisting hypertension and there were no abnormalities in other vital signs or blood samples during the trial period. Although an open label trial is not free from practice effects or rater–caregiver expectations, statistically significant differences between baseline and 6 weeks treatment were a reduction in neuropsychiatric symptoms and an improvement in attention.

Keywords: *S. lavandulaefolia*; Essential oil; Alzheimer's disease; Tolerability; Monoterpenoids; Anticholinesterase; Antioxidant; Anti-inflammatory; Oestrogenic

1. Introduction

The majority of FDA-approved drugs for Alzheimer's disease (AD) (e.g., tacrine, donepezil, rivastigmine, galantamine) act by countering the cholinergic deficit associated with the cognitive dysfunction and are based on inhibition of the enzyme acetylcholinesterase (AChE) (Giacobini, 2000, 2001; Talesa, 2001). Peripheral cholinergic (gastrointestinal) adverse effects for currently used cholinesterase inhibitors are common as well as other side effects such as hepatotoxicity (tacrine). More recently, the uncompetitive NMDA (*N*-methyl-D-aspartate) antagonist memantine that improves functioning and behavioural symptoms in patients with AD has been approved (Helmuth, 2002). Other targets include anti-inflammatory, antioxidative and oestrogenic mechanisms, nicotinic receptors, nerve growth factors and the formation of neurofibrillary tangles and Aβ plaques—the hallmarks of the diseased brain (Cutler and Sramek, 2001; Fluck et al., 2000; Wolfe, 2001; Zandi and Breitner, 2001). It is increasingly evident that preventative and symptomatic treatment of AD will become a multitarget drug strategy.

The premise of traditional herbal medicine is treatment of the whole disorder as opposed to single isolated symptoms of the disease. Numerous herbal extracts, containing several active constituents and often more than one plant species, have been used to treat CNS-related disorders. Amongst these, *Salvia* species (*S. officinalis* L. and *S. lavandulaefolia* Vahl., *S. miltiorrhiza* Bung.) are prominent for their reputed beneficial effects on memory disorders, depression and cerebral ischaemia (Howes et al., 2003; Perry et al., 2000a,b). Several studies have now been undertaken to scientifically investigate the traditional (CNS-related) use of *S. lavandulaefolia* (a sage species that contains only trace

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amounts of the convulsant thujone) relevant to the treatment of Alzheimer's disease; these have centred on the activity of the essential (volatile) oil (Perry et al., 1996, 2000, 2001, 2002; Savelev et al., 2003).

2. Pharmacological activities of *Salvia lavandulaefolia* relevant to Alzheimer's disease

2.1. Antioxidant

Many *Salvia* species and their isolated constituents possess significant antioxidant activity in enzyme-dependent and enzyme-independent systems (Dorman et al., 1995; Hohmann et al., 1999; Lu and Foo, 2001; Malencic et al., 2000; Zupko et al., 2001). A number of these have exhibited effects relevant to potential treatment of CNS related disorders (Howes et al., 2003), the flavonoid apigenin for example has been shown to protect neurons against Aβ induced toxicity (Wang et al., 2001). *S. lavandulaefolia* ethanolic extracts (both the water-soluble and chloroform-soluble fractions), individual constituents of the essential oil [the monoterpenoids 1,8-cineole (1), linalool (5), α(2)- and β-pinene] and herb [the phenolic monoterpenoid carvacrol (7), the flavone luteolin (8) and the phenolic rosmarinic acid (10)] have been reported to be antioxidant (Adam et al., 1998; Dorman et al., 1995; Lu and Foo, 2001; Malencic et al., 2000; Perry et al., 2001; Zupko et al., 2001) while camphor (20–30% of essential oil) has demonstrated pro-oxidant effects in a liposome peroxidation preparation (Perry et al., 2001) (Table 1). The herb constituent rosmarinic acid displays more potent radical scavenging activity than trolox (a derivative of α-tocopherol) (Lu and Foo, 2000, 2002) and the inhibition of liposome peroxidation by the monoterpenoids is considered weak, though significant compared to the standard antioxidant propyl gallate (Perry et al., 2001). Given that the monoterpenoids with antioxidant activity in this study were present in the essential oil at a slightly higher relative percentage (collectively over 30%) than camphor (27% of essential oil), the pro-oxidant activity of camphor may not have its effect in the whole essential oil. Further to this any potential synergistic or antagonistic interactions could change the antioxidant profile of a whole extract and further studies, using other systems, should be undertaken as potential antioxidant activity can depend on the systems used and may also depend on concentration (Motohashi et al., 2002).

2.2. Anti-inflammatory activity

In addition to antioxidant activity many *Salvia* species and their isolated constituents demonstrate anti-inflamma-

Table 1
In vitro activities of extracts and constituents of *S. lavandulaefolia* relevant to the treatment of Alzheimer's disease

Constituent (% essential oil)	Antioxidant[a]	Anti-inflammatory[b]	Oestrogenic[c]	AntiChE[d] (IC50)	Sedative/CNS depressant[e]
EtOH extract	✓5 mg/ml	✓50 μg/ml	✓1.25 mg/ml	–	–
$CHCl_3$ fraction	✓5 mg/ml	✓50 μg/ml	×	–	–
H_2O fraction	✓5 mg/ml	✓50 μg/ml	✓5 mg/ml	–	–
Essential oil	–	–	✓0.13 μg–1.3 mg/ml	✓0.03 mg/ml	–
1,8-Cineole (1) (15–30%)	✓0.1 M	×0.2 M	×0.001–2.25 mM	✓0.4–7 mM	–
α-Pinene (2) (4–7%)	✓0.1 M	✓0.2 M	×0.001–2.25 mM	✓0.67 mM	–
β-Pinene (5–12%)	✓0.1 M	–	×0.001–2.25 mM	✓1.5 mM	–
Borneol (3–15%)	–	–	–	✓>2 mM[e]	–
Camphor (3) (20–30%)	× pro-oxidant	×0.2 M	×[e]	✓>5 mM	–
Geraniol (4) (<1%)	×	✓0.2 M	✓0.1–2 mM	✓>5 mM	–
α-Terpineol (6) (<1%)	–	–	–	✓~5 mM	✓[e] (in vivo)
γ-Terpinene (<1%)	–	–	–	✓>5 mM	–
Caryophyllene epoxide (10)	–	–	–	✓>2 mM[e]	–
Linalool (5) (<1%)	✓[e]	–	–	✓>5 mM[e]	✓[e]
Carvacrol (7)	✓[e]	✓[e]	–	–	–
Rosmarinic acid (10)	✓[e]	✓[e]	–	–	–
Luteolin (8)	✓[e]	✓[e]	–	–	–
Genkwarin	–	✓[e]	–	–	–
Cirsimartin	–	✓[e]	–	–	–
Salvigenin	–	✓[e]	–	–	–
Cirsiliol (11)	–	–	–	–	✓[d]

– = not assessed.

Unless otherwise stated, data are taken from Perry et al. (1996, 2000b, 2001).

[a] Inhibition of lipid peroxidation in bovine phospholipid liposomes.

[b] Inhibition of formation of TXB_2 and LTB_4 (calcium-ionophore-stimulated rat leucocytes).

[c] Oestrogenic activity using a recombinant oestrogen-inducible screen in yeast.

[d] Inhibition of bovine erythrocyte AChE using colorimetric assay of Ellman et al. (1961).

[e] Adam et al., 1998; Atanasovo-Shopova et al., 1973; Buchbauer et al., 1993; Dorman et al., 1995; Lu and Foo, 2001, 2002; Malencic et al., 2000; Paladini et al., 1999; Silva Brum et al., 2001; Tinwell et al., 2002; Zupko et al., 2001).

tory properties (Bingol and Sener, 1995; Howes et al., 2003; Maklad et al., 1999) (Table 1). Carvacrol (7) and α-pinene have been shown to be anti-inflammatory constituents of *S. lavandulaefolia* (Bingol and Sener, 1995; Wagner et al., 1986), in addition, the latter monoterpenoid has been shown to inhibit the enzyme cycloxygenase, an activity that may be of particular relevance to anti-inflammatory treatment of AD (Zandi and Breitner, 2001). Other constituents that have shown anti-inflammatory activity in various in vitro assays are the flavones genkwarin and luteolin (8) and the 6-hydroxy flavones cirsimaritin and salvigenin and several polyphenolics including rosmarinic acid (10) (Bingol and Sener, 1995; Wagner et al., 1986). *S. lavandulaefolia* ethanolic extracts (the chloroform-soluble fraction over the water-soluble fraction) and monoterpenoids present in the essential oil, α-pinene (2) and geraniol (4), have demonstrated weak (significant) inhibition of eicosanoid synthesis, though there may be more potent constituents present in minute quantities in the essential oil (Perry et al., 2001). The effect of the total oil would be expected to be weak, but in favour of inhibition of 5-lipoxygenase inhibition, since α-pinene (5% of essential oil tested) showed weak selectivity for the pro-inflammatory eicosanoid leukotriene B_4 (LTB_4), whereas geraniol (<1% of essential oil) showed weak selectivity for thromboxane B_2 (Perry et al., 2001). LTB_4 is produced via the enzyme 5-lipoxygenase, the gene for which is up-regulated during neurodegeneration and although the role of this inflammatory mediator in AD is not entirely apparent, selective inhibition may be therapeutically relevant (Sugaya et al., 2000). Screening in other anti-inflammatory assays that use mediators more specific to AD would be valuable, such as the classic complement cascade surrounding the site of Alzheimer amyloid plaques and inhibition of interleukin-1β and the inflammatory cytokine S-100β, levels of which are also increased in the Alzheimer brain (Zandi and Breitner, 2001).

2.3. Oestrogenic activity

In addition to antioxidant and anti-inflammatory activity, dose-dependent oestrogenic activity was demonstrated in an ethanolic extract (which appeared to be concentrated in the water-soluble fraction) of *S. lavandulaefolia* (Perry et al., 2001). The essential oil and constituent monoterpenoid geraniol (<1% of essential oil) also showed oestrogenic activity, the latter of which was dose dependent and weak in comparison to the standard oestrogenic compound, 17β-oestradiol (Table 1). The monoterpenoid camphor (3) was shown to be inactive in yeast oestrogen receptor transactivation assay (Tinwell et al., 2002). The potential oestrogenic activity of the *S. lavandulaefolia* extracts, essential oil and constituents requires further investigation in an in vivo environment where metabolic processes and the effectiveness of a compound-receptor complex in producing a gene response may alter oestrogenic activity (Green et al., 1998).

2.4. Cholinesterase inhibition

A further activity, and one that originally stimulated research into the putative CNS beneficial effects of *S. lavandulaefolia*, is its ability to inhibit AChE. *S. lavandulaefolia* essential oil inhibits human brain (autopsy tissue) AChE in vitro (IC50 0.07 mg/ml) (Perry et al., 1996) and bovine erythrocyte AChE in vitro (IC50 0.03–5 mg/ml) (Perry et al., 2000b; Savelev et al., 2003). This inhibition has been confirmed in vivo; oral administration of *S. lavandulaefolia* essential oil once daily for 5 days to rats resulted in decreased striatal AChE activity at a lower dose and decreased striatal and hippocampal AChE activity at a higher dose; at both doses there was no change in the AChE activity in the cortex (Perry et al., 2002). Thus, it is apparent that following oral administration to rats one or more constituents of *S. lavandulaefolia* essential oil or their metabolites, reach the brain (crossing the gastrointestinal and blood–brain barriers) to inhibit AChE in select brain areas, consistent with evidence of inhibition of the brain enzyme in vitro.

The major (commercially obtained) monoterpenoid constituents of *S. lavandulaefolia* essential oil inhibit bovine erythrocyte AChE with varying degrees of potency (Table 1) (Miyazawa et al., 1997; Perry et al., 2000b; Ryan and Byrne, 1988; Savelev et al., 2003). 1,8-Cineole (15–30% of essential oil) and α-pinene (4–7%) show the most inhibition (IC50 0.4–0.7 mM) that is less active by factor of at least 10^3 compared to the anticholinesterase (antiChE) alkaloid physostigmine. It was assumed that the antiChE activity of the major monoterpenoid constituents (camphor, 1,8-cineole, borneol α- and β-pinene) were responsible for the antiChE activity of the whole essential oil. However, constituents combined in a naturally occurring ratio were significantly less potent than that of the whole oil (Perry et al., 2000b; Savelev et al., 2003). It was proposed that the monoterpenoids may act synergistically to inhibit AChE and a recent study (Savelev et al., 2003) examined putative interactions (synergism, antagonism) or zero-interaction between the antiChE constituents of *S. lavandulaefolia* essential oil. It was concluded that the inhibitory activity of *S. lavandulaefolia* essential oil results from complex interactions, which produce both synergistic and antagonistic responses between the constituent terpenoids. For example, minor synergy was found between 1,8-cineole (1) and α-pinene (2) and 1,8-cineole (1) and caryophyllene epoxide (10) and antagonism was found to occur between 1,8-cineole (1) and camphor (3).

This antiChE activity *S. lavandulaefolia* essential oil terpenoid constituents is of interest since previously identified antiChEs are amines, the naturally occurring type being alkaloids and these compounds were not known to exist in the *Salvia* species tested. Other nonmonoterpenoid compounds present in *S. lavandulaefolia* herb may inhibit AChE since the disesquiterpene gossypol (Ryan and Byrne, 1988), the coumarin auraptene and the sesquiterpenoids nookatone (Howes et al., 2003) and caryophyllene epoxide (10) (1% *S.*

lavandulaefolia essential oil) (Savelev et al., 2003) are also weak inhibitors of AChE.

2.5. Tolerability trial of S. lavandulaefolia essential oil in Alzheimer's disease

Since the above effects of *S. lavandulaefolia* (essential oil) are currently relevant to treatment of AD and a recent placebo-controlled, double-blind, balanced, cross-over design study of *S. lavandulaefolia* in healthy volunteers (Kennedy et al., 2002) demonstrated that the essential oil produced a number of significant effects on cognition (including improvements in both immediate and delayed word recall scores that were coupled with decrements in both accuracy and speed of attention and were associated with reductions in self-rated 'alertness' and 'calmness') clinical trials in AD are indicated. It was considered initially important to assess tolerability in patients with AD. A (phase-II) pilot open-label trial was carried out which, although there was no placebo control, also endeavoured to determine potential efficacy and to identify appropriate drug dose ranges in order to maximize the potential for demonstrating efficacy in subsequent extended clinical trials. This involved a limited number of patients ($n = 11$) for a short period of time (6 weeks).

3. Methods

3.1. Patient cohort

The trial (conducted in 1999 in Newcastle Upon Tyne, U.K.) included 11 patients (1 male and 10 female), aged 76–95 years in whom a diagnosis of mild to moderate probable Alzheimer's disease (NINCDS/ADRDA criteria; McKhann et al., 1984) had been established. Patients had a MMSE (Mini-Mental State Examination) score of between 10 and 26 and Neuropsychiatric Inventory (NPI) scores for items 3 and 9 were 0. All patients had vision and hearing sufficient for compliance with testing procedures. The study received Newcastle Joint Ethical Committee approval, and all patients and their carers were fully informed and provided written and oral consent. Use of other drugs continued (exceptions below) with no change in prescription during the trial period. Nine patients were in residential homes, two living at home—one was capable of taking medication herself and the other was living with her daughter.

3.1.1. Exclusion

Patients with the following were excluded: baseline heart rate below 50; use of tricyclic antidepressants, thioridazine or related neuroleptic; investigative drugs taken in the last 4 weeks; cholinergic therapy within the previous 6 months (and known hypersensitivity to cholinesterase inhibitors); clinically significant gastrointestinal, renal, hepatic, endocrine, pulmonary, neurological (e.g., epilepsy, stroke) or cardiovascular (myocardial infarction in previous 3 months) diseases; haematological or oncological disorder within previous 2 years, history of alcohol or drug abuse, vitamin B12 or folate deficiency, alanine transaminase >180 U/l, haemoglobin <10, white blood cell <4, creatinine >180; a high bp (>100 mm Hg diastolic). Also because of the possible hypoglycaemic effect of sage (Zarzuelo et al., 1990) patients with any form of diabetes were excluded.

3.2. Intervention

3.2.1. Extract

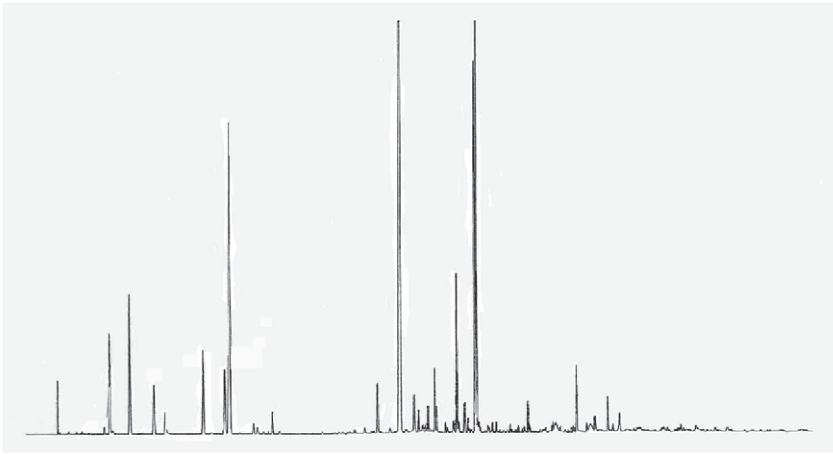
Capsules contained 50 μl essential oil of *S. lavandulaefolia* plus 50 μl of sunflower oil (*Helianthus annuus*) (as a carrier) and were maintained at 4 °C. The essential oil was commercially extracted by Advanced Phytonics (Olway Works, Healey Road, Ossett, WF5 8LT, U.K.) from the flowering tops of the plant (grown in Granada, Spain and harvested in early Spring of 1999) and encapsulated by Power Health (Poklington, U.K.). The gas chromatography profile of the capsulated material (analysed 5 months after capsulation, by the Scottish Agricultural Council, Auchincruive, Ayr, Scotland) showing the main constituents of the essential oil is provided in Fig. 1 [BP20 column (25 m × 0.25 mm; film thickness, 0.25 μm; with polar, bonded stationary phase of polythene glycol); 50–200 °C at 5 °C min^{-1} + 10 min; injection and FID temperature, 250 °C; injection volume, 200 μl]. GC analysis demonstrated 94 peaks, the major peaks were of borneol, camphene, camphor (3), 1,8-cineole (1) and α-terpineol (6), with only a trace of thujone consistent with that in literature (Fournier et al., 1993).

3.2.2. Dosage

Week 1: one capsule at 8 a.m.; Week 2: one capsule at 8 a.m. and one capsule at 7 p.m.; Weeks 3–6: as above with one additional capsule at 12:30 p.m. Capsules were dispensed in weekly amounts in dark glass bottles with an audit of capsules taken by pill counts, checking medication sheets and questioning the caregiver at each visit.

3.3. Study design

Vital signs (weight, physical and neurological examination and heart rate), adverse events, capsule counts and drug dispensation were recorded at the end of every week. Complete assessments of primary outcome measures were conducted at baseline and after 6 weeks of treatment. At screening and at termination physical and extensive neurological examinations were carried out, as well as complete laboratory tests. Blood samples were screened for B12, red cell folate, free T4, TSH, sodium, potassium, chloride, calcium, phosphate, urea, creatinine, albumin, alkaline phosphatase, bilirubin, plasma glucose, cholesterol, ALT, full blood count, number and state of red blood cells (RBC) (haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration,



Monoterpenoid constituent*	Retention time (min)	Relative percentage peak† (% of essential oil)
camphene	6.84	4.0
camphor (3)	24.81	29.6
caryophyllene epoxide (10)	27.05	1.5
1,8-cineole (1)	13.52	12.7
borneol	29.69	10.3
limonene	13.15	2.2
myrcene	11.73	2.6
α-terpineol (6)	29.83	10.1
α-pinene (2)	5.53	2.2
β-pinene	8.46	1.7

*Identities of compounds determined from the retention times

† Relative percentages as internal normalisation of total peaks observed.

Fig. 1. Gas chromatographic profile of the major constituents of *S. lavandulaefolia* essential oil used (BP20 column; 50–200 °C at 5 °C min^{-1} + 10 min; injection and FID temperature 250 °C; injection volume 200 μl).

platelets, mean plasma volume, human calcitonin) and AChE. Blood was collected in heparinised tubes, spun, plasma withdrawn and stored at −20 °C until analysed. RBC AChE and plasma butyrylcholinesterase (BuChE) activity was assessed using a modified version of the colorimetric method of Ellman et al. (1961; see Perry et al., 2000b) and the catalytic concentration of enzyme (*b*) expressed in units per litre (U/l) using the formula: b (U/l) = $F./\epsilon \times \Delta A/.\Delta t$; where F = dilution factor for the RBC in sodium phosphate buffer; ϵ = extinction coefficient in $mM^{-1}cm^{-1}$; ΔA = absorbance per minute.

3.4. Outcome measures

MMSE, performed at baseline and 6 weeks, is a simplified form of the cognitive mental status examination suitable for AD patients and assesses response to drug treatment and includes 11 questions (test time 5–10 min) (McShane et al., 1997; Folstein et al., 1975). The Cognitive Drug Research (CDR) computerised cognitive assessment system is a validated, well-tolerated assessment that is sensitive to drug-induced cognitive performance change (Simpson et al., 1988) and assesses performance on a number of tests including word presentation, immediate word recognition and simple reaction time. NPI, developed to assess psychopathology in dementia patients evaluates 12 neuropsychiatric disturbances common in dementia including delusions, hallucinations, agitation, depression, anxiety and also appetite and eating changes in patients and is sensitive to treatment effects. Paired samples t tests were used to assess posttreatment differences from baseline for all the assessments; P values of .05 or less were considered significant.

4. Results

4.1. Tolerability

There were no patient withdrawals during the trial and no patient experienced any adverse physical or neurological effects throughout the study. Blood samples, taken at the end of the trial, showed no statistically significant changes in

any measure in any patient. Although not statistical significant, there was an increase in bilirubin after 6 weeks treatment, the mean increase between values before and after treatment was 1.30 μmol/l (P=.057). Blood pressure was measured weekly and there were no changes in 9 of the 11 patients. In two patients with a history of hypertension, there were increases in blood pressure at 3 weeks (highest dose); diastolic rose to 100 and 110 mm Hg and systolic rose, in only one of these, to 200 mm Hg which when included in total analysis gave a mean increase (n = 11) in diastolic bp of 15.09 (P=.025) and systolic bp of 6.19 (P=.049).

4.2. Blood cholinesterases

Blood cell samples were screened for RBC AChE and plasma BuChE and although results were variable there was a mean decrease (14.4%) in AChE activity as a result of treatment (Table 2). Mean BuChE activity showed no overall change (Table 2), with seven patients showing a decrease (3635.6 ± 634.8 to 3354 ± 477.8 U/l) and four showing an increase (2793 ± 651.9 to 3232.3 ± 907.5 U/l). In vitro (bovine erythrocyte) AChE inhibition by the same batch of *S. lavandulaefolia* essential oil gave an IC50 value 0.116 μl/ml.

4.3. Clinical outcome measure

Although the trial was not originally designed to assess efficacy in terms of cognitive function or behavioural features, the following observations were made. The mean MMSE for the whole group remained statistically the same between baseline and 6 weeks (Table 2). The CDR results indicated no apparent adverse effects on cognition and there were several interesting trends indicating an improvement in memory and attention that in one instance reached statistical significance (Table 2). Thus, using a one-tailed paired t test (with level of significance set at .01 in view of the multiple tests), vigilance task performance (VIGACC; detection of target digits) improved (P=.01). However, there were also significantly more false alarms (i.e., responses when the target stimuli were not present) made during the number vigilance test (VIGFA; P=.03). There was also a trend for improvement on an immediate word recognition task (P=.14). There was a statistically significant improvement in the NPI measured after 6 weeks compared to baseline (P=.024; Table 2) and this was also evident in the patient with the highest blood pressure.

Table 2
Mean differences in demographics and outcome measures following oral administration of *S. lavandulaefolia* essential oil

Assessment	Baseline mean ± S.D. (x–y)	After treatment[a] mean ± S.D. (x–y)
MMSE	19.36 ± 4.20 (10–26)	19.82 ± 3.52 (14–26)
NPI	5.70 ± 6.25 (0–20)	2.70 ± 5.29 (0–16)[b]
CDR		
VIGACC[c]	81.21 ± 35.50 (7–100)	83.70 ± 32.34 (27–100)[d]
VIGFA[c]	1.91 ± 3.24 (0–11)	3.44 ± 4.93 (0–14)
IRECSD[c]	523 ± 412 (22–1495)	972 ± 914 (108–3142)
IRECRTM[c]	1748 ± 1322 (470–1700)	2054 ± 1319 (518–5574)
AChE (U/L)[e]	16,765 ± 4808 (10,268–20,952)	14,353 ± 5048 (9939–26,182)
BuChE (U/L)[e]	3329 ± 742 (1988–4592)	3310 ± 623 (2102–4308)

Data are shown as mean ± S.D. (data range).

[a] *S. lavandulaefolia* essential oil (6 weeks).

[b] P=.0243; t = 2.324.

[c] VIGACC = vigilance task: ability to respond to target digits; VIGFA = vigilance task: false alarms; IRECSD= immediate recognition standard deviation; IRECRTM = immediate recognition median reaction time.

[d] P=.014; t = 2.684.

[e] Catalytic units/l = $F./\epsilon \times \Delta A/.\Delta t$; where F = dilution factor for the RBC in sodium phosphate buffer; ϵ = extinction coefficient in $mM^{-1}cm^{-1}$; ΔA = absorbance per minute.

5. Discussion

This is the first open-label trial to evaluate the tolerability of oral administration of *S. lavandulaefolia* essential oil in patients with AD. In addition to *S. lavandulaefolia* essential oil proving safe at this dosage (patients, with the exception of the two with a history of hypertension, did not experience any side effects while taking the capsules) there were significant improvements in NPI and CDR scale assessment. This is remarkable given the small heterogenous sample size, diverse MMSE scores and the variability in performance associated with a dementia population. Given that two patients experienced an increase in blood pressure (and since the patient population was heterogenous and limited) investigation into the long-term effects of *S. lavandulaefolia* essential oil on blood pressure would be appropriate. The fact that sage (species not specified) should not be administered to people with high blood pressure is recorded in one of the medical herbals (Bartram, 1995) and in future efficacy studies such patients (with a history of hypertension) should be excluded or at least monitored and withdrawn from the trial in the event of increased blood pressure. Although this side effect may impact the ultimate effectiveness of *S. lavandulaefolia* essential oil as a potential treatment for AD, the increase in blood pressure in two patients coincided with administration of the highest dose of (50 μl 3 ×/day). An equivalent dose of herb (*S. lavandulaefolia* leaf containing approximately 2% of essential oil) would be 2.5 g 3 × per per day and in future trials a dose less than 50 μl 3 ×/day is likely to be the most appropriate since a lower dose of essential oil has been shown to have cognitive effects in young volunteers (Tildesley et al., 2003). In addition the whole herb may have different/ no blood pressure effects and a better and larger selection of patients may clarify the effects of *S. lavandulaefolia* on hypertension.

These initial clinical data demonstrate that *S. lavandulaefolia* essential oil results in improvements in memory and

attention in patients with AD and improves memory in healthy volunteers (Kennedy et al., 2002) and this corroborates the use of sage (*S. lavandulaefolia*) for memory related disorders recorded in English medical herbals (Perry et al., 2000a).

While a placebo controlled trial is now required, these findings together with those of Tildesley et al. (2003) demonstrate that following oral administration of *S. lavandulaefolia* essential oil significant cognitive or behavioural effects can be observed in human subjects. Although it is possible that peripheral side effects, practice and rater–caregiver expectations could play a role, it can be suggested that *S. lavandulaefolia* constituents and/or their metabolites reach the brain (crossing the gastrointestinal and blood–brain barriers) and exert an effect on cognition. The biochemical/neuronal systems affected are likely to include the cholinergic system since the essential oil constituents inhibit erythrocyte AChE in vitro (Perry et al., 2000b) and rat brain AChE in vivo (Perry et al., 2002) and although results require duplication, blood samples from the present study suggest a trend towards inhibition of erythrocyte AChE following oral administration in humans. In addition, the glutamatergic system may be involved in the CNS effects of *S. lavandulaefolia* since the monoterpenoids linalool (5) and terpineol (together < 11% of the essential oil), have been shown to have in vivo CNS depressant action (Atanasovo-Shopova et al., 1973; Buchbauer et al., 1993), and linalool (5) has been shown to competitively antagonise [^{3}H]glutamate binding to receptors (Silva Brum et al., 2001) (Table 1). An effect of other constituents of the essential oil on glutamatergic and GABAergic systems cannot be excluded since thujone (present only in trace quantities in *S. lavandulaefolia*) has been shown to competitively antagonise $GABA_A$ receptor agonists (Hold et al., 2000) and camphor (3) (20–30% of essential oil) has convulsive effects at high doses (Emery and Corban, 1999). *Salvia* species are widely used for their anxiolytic and sedative properties and this use is supported by in vitro and in vivo data that show a structurally diverse number of constituents [e.g., caffeic acid ethyl esters, cirsiliol (11), carnosic acid, miltirone, linalool (5)] act as CNS depressants at the GABA chloride channel and/or at glutamate binding sites (Chang et al., 1991; Dentali and Hoffman, 1990; Silva Brum et al., 2001; Maklad et al., 1999; Marder et al., 1996; Rutherford et al., 1992; Silva Brum et al., 2001; Paladini et al., 1999). Cirsiliol (11), one of many plant flavonoids that have been shown to have affinity for the benzodiazepine receptor site (Ki 200 μM) (Paladini et al., 1999), is present in *S. lavandulaefolia* herb and is unlikely to be present in the essential oil. It is thus worth considering a combination of herb extract and essential oil in future studies since potential antioxidant [carvacrol (7)], anti-inflammatory [rosmarinic acid (9), genkwarin, luteolin (8), cirsimartin and salvigenin] and other CNS-active constituents [cirsiliol (11)] are likely to be present in whole herb extracts (Table 1, Fig. 2). It is important in future studies to determine the mechanism of action of (monoterpenoid) constituents present in essential oils in general, since constituents and/or metabolites enter the blood, cross the blood–brain barrier and reach the brain to have an observable CNS effect following inhalation and/or topical application and since they are used in aromatherapy

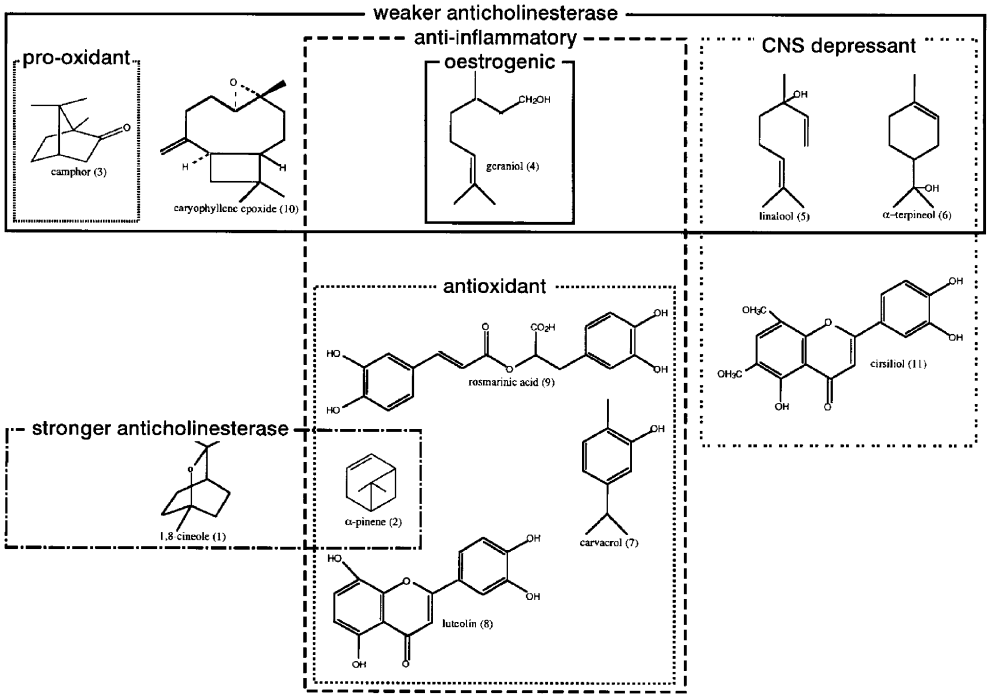


Fig. 2. Constituents present *S. lavandulaefolia* and their putative pharmacological activities.

worldwide (e.g., Atanasovo-Shopova et al., 1973; Buchbauer et al., 1993; Buckle, 1993; Jirovetz et al., 1990; Yamada et al., 1994).

Thus, although evidence so far indicates that *S. lavandulaefolia* may be appropriate to the treatment of AD (Fig. 2), further in vitro and in vivo studies are necessary to determine structure–activity relationships and pharmacological interactions between (monoterpenoid) constituents responsible for the observed effect. For example, in addition to the potential interactive (antagonistic) antiChE effect of the monoterpenoid camphor (20–30% of essential oil) (Savelev et al., 2003), camphor has shown pro-oxidant effects in one study (Perry et al., 2001) and is associated with hepato- and neurotoxicity at high doses (Aliye et al., 2000; Jimenez et al., 1983). It could be recommended that this constituent be extracted from the essential oil in further studies. A further example of the variability of in vitro (isolated) constituent effects compared to in vivo (combined) effects is that a thujone 7-hydroxy metabolite attains much higher brain levels than α-thujone (the naturally occurring form) and is less toxic to mice and less potent in a $GABA_A$ receptor binding study (Hold et al., 2000). In addition information is needed on the pharmacokinetics of *S. lavandulaefolia* constituents—how they are metabolised, what the maximum duration of action and half-life is and further how they may interact with other drugs since monoterpenoids have been shown to inhibit enzymes of the cytochrome P4502B subfamily (De-Oliveira et al., 1997). Issues concerning dosage and extract form (herb and/or essential oil) are complex. Complete correlation of the relative percentage of active constituents in preparations with the knowledge of pharmacological interactions and biological effects may well be impractical based on present knowledge. Future trials should therefore endeavour to control for variations in the chemical constituents and provide quantitative chemical and bioactive profiles in all reported studies.

6. Conclusion

S. lavandulaefolia has pharmacological activities relevant to dementia therapy. In a pilot clinical trial, after 6 weeks of treatment with *S. lavandulaefolia* essential oil in patients with AD, statistically significant differences included an improvement in accuracy for the detection of target digits (VIGACC), an increase in the number of false alarm responses during the number of vigilance test (VIGFA) and a lower NPI score on both main items. Patients did not experience any adverse effects while taking *S. lavandulaefolia* essential oil capsules although there was an increase in systolic and diastolic blood pressure in patients with predisposing hypertension[1].

[1] During the preparation of this paper a 4-month placebo-controlled clinical trial of a *salvia officinalis* extract demonstrating significant improvement in cognition in mild/moderate Alzheimer's disease (19 on sage, 20 on placebo) has been published, (Akhondzadeh et al., 2003).

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References

Adam JN, Sivropoulou A, Kokkini S, Lanaras T, Arsenakis M. Antifungal activities of *Origarnum vulgare* subsp. *Hirtum*, *Mentah spicata*, *Lavandula angustifolia* and *Salvia fruticose* essential oils against human pathogenic fungi. J Agric Food Chem 1998;46:1739–45.

Akhondzadeh S, Noroozian M, Mohammadi M, Ohadinia S, Jamshidi AH, Khani M. *Salvia officinalis* extract in the treatment of patients with mild to moderate Alzheimer's disease: a double blind, randomized and placebo-controlled trial. J Clin Pharm Ther 2003;28(1):53–9.

Aliye UC, Bishop WP, Sanders KD. Camphor hepatotoxicity. South Med J 2000;93(6):596–8.

Atanasovo-Shopova S, Rusinov KS, Boicheva I. Central neurotropic effects of lavender essential oil: II. Effects of linalool and terpineol. Chem Abstr 1974;81:58351.

Bartram T. Encyclopaedia of herbal medicine. 1st ed. London: Grace; 1995. p. 379.

Bingol LM, Sener B. A review of terrestrial plants and marine organisms having anti-inflammatory activity. Int J Pharmacogn 1995;33(2):81–97.

Buchbauer G, Jirovetz L, Jager W, Plant C, Dietrich H. Fragrance compounds and essential oils with sedative effects upon inhalation. J Pharm Sci 1993;82(6):660–4.

Buckle J. Aromaratherapy. Nurs Times 1993;89(20):32–5.

Chang HM, Chui KY, Tan FWL, Yang Y, Zhong ZP. Structure–activity relationship of miltirone, an activi benzodiazepine receptor ligand isolated from S*alvia miltiorrhiza* Bunge (Danshen). J Med Chem 1991;34:1675–92.

Cutler N, Sramek JJ. Review of the next generation of Alzheimer's disease therapeutics: challenges for drug development. Prog Neuropsychopharmacol Biol Psychiat 2001;25:24–57.

Dentali SJ, Hoffman JJ. 16-Hydroxycarnosic acid, a diterpene from *Salvia apiana*. Phytochemistry 1990;29(3):993–4.

De-Oliveira ACAX, Ribeiro-Pinto LF, Paumgartten FJR. In vitro inhibition of CYP2B1 monooxygenase by β-myrcene and other monoterpenoid compounds. Toxicol Lett 1997;92:39–46.

Dorman HJP, Deans SG, Noble RC. Evaluation in vitro of plant essential oils as natural antioxidants. J Essent Oil Res 1995;7:645–51.

Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88–95.

Emery DP, Corban JG. Camphor toxicity. J Paediatr Child Health 1999;35:105–8.

Fluck E, File SE, Rymer J. Ten years of hormone replacement therapy and

performance in tasks associated with frontal lobe function. Br J Clin Pharmacol 2000;50:384–5.
Folstein MF, Folstein SE, McHugh PR. ''Mini Mental State'', a practical method for grading the cognitive state of patients for the clinician. J Psychiat Res 1975;12:189–98.
Fournier G, Pages N, Casperec I. Contribution to the study of *Salvia lavandulaefolia* essential oil: potential toxicity attributable to sabinyl acetate. Planta Med 1993;59:96–7.
Giacobini E. Cholinesterase inhibitors: from Calabar bean to Alzheimer therapy. In: Giacobini E, editor. Cholinesterases and cholinesterase inhibitors. London: Martin Dunitz; 2000. p. 181–226.
Giacobini E. Do cholinesterase inhibitors have disease-modifying effects in Alzheimer's disease? CNS Drugs 2001;15(2):85–91.
Green PS, Gridley KE, Simpkins JW. Nuclear estrogen receptor-independent neuroprotection by estratrienes: a novel interaction with glutathione. Neuroscience 1998;84:7–10.
Helmuth L. New therapies. New Alzheimer's treatment that may ease the mind. Science 2002;297(5585):1260.
Hohmann J, Zupko I, Redei D, Csanyi M, Falkay G, Mathe I, et al. Protective effects of the aerial parts of *Salvia officinalis*, *Melissa officinalis*, and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-indepenent lipid peroxidation. Planta Med 1999; 65:576–8.
Hold KM, Sirisoma NS, Ikeda T, Narahashi T, Casida JE. α-Thujone (the active component of absinthe): γ-Aminobutyric acid type A receptor modulation and metabolic detoxification. PNAS 2000;97(8):3826–31.
Howes M-JR, Perry NSL, Houghton PJ. Plants with traditional uses and activities relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother Res 2003;17(1):1–18.
Jimenez JF, Brown AL, Arnold WC, Byrne WJ. Chronic camphor ingestion mimicking Reye's syndrome. Gastroenterology 1983;84:394–8.
Jirovetz L, Buchbauer G, Jager W, Raverdino V, Nikiforov A. Determination of lavender oil fragrance compounds in blood samples. Fresenius J Anal Chem 1990;338:922–3.
Lu Y, Foo LY. Antioxidant activities of polyphenols from sage (*Salvia officinalis*). Food Chem 2001;75:197–202.
Lu Y, Foo LY. Polyphenolics of *Salvia*—a review. Phytochemistry 2002;59: 140–77.
Maklad YA, Aboutabi EA, El-Sherei MM, Meselhy KM. Bioactivity studies of *Salvia transsylvannica* (Schur ex Griseb) grown in Egypt. Phytother Res 1999;13:147–50.
Malencic DJ, Gasic O, Popovic M, Boza P. Screening for antioxidant properties of *Salvia reflexa* Hornem. Phytother Res 2000;14:546–8.
Marder M, Viola H, Wasowski C, Wolfman C, Waterman PG, Medina JH, et al. Cirsiliol and caffeic acid ethyl ester, isolated from *Salvia guaranitica* are competitive ligands from the central benzodiazepine receptors. Phytomedicine 1996;3(1):29–31.
McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadian EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 1984;34:939–44.
McShane R, Keene J, Fairburn C, Jacoby R, Hope T. Issues in drug treatment for Alzheimer's disease. Lancet 1997;350:886–7.
Miyazawa M, Watanabe H, Kameoka H. Inhibition of acetylcholinesterase activity by monoterpenoids with a *p*-menthane skeleton. J Agric Food Chem 1997;45:677–9.
Motohashi N, Shirataki Y, Kawase M, Tani S, Sakagami H, Satoh K, et al. Cancer prevention and therapy with kiwifruit in Chinese folklore medicine: a study of kiwifruit extracts. J Ethnopharmacol 2002;81:357–64.
Paladini AC, Marder M, Viola H, Wolfman C, Wasowski C, Medina JH. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol 1999;51:519–26.
Perry N, Court G, Bidet N, Court J, Perry E. European herbs with cholinergic activities: potential in dementia therapy. Int J Geriatr Psychiatry 1996;11:1063–9.
Perry N, Howes M-J, Houghton P, Perry E. Why sage may be a wise remedy: effects of *Salvia* on the nervous system. In: Kintzios SE, editor. Sage: the genus *Salvia*. Netherlands: Harwood; 2000a. p. 207–23.
Perry NSL, Houghton PJ, Theobald AE, Jenner P, Perry EK. In-vitro inhibition of erythrocyte acetylcholinesterase by *Salvia* lavandulaefolia essential oil and constituent terpenes. J Pharm Pharmacol 2000b;52: 895–902.
Perry NSL, Houghton PJ, Sampson J, Theobald AE, Hart S, Lis-Balchin M, et al. In-vitro activity of *S. lavandulaefolia* (Spanish sage) relevant to treatment of Alzheimer's disease. J Pharm Pharmacol 2001;53: 1347–56.
Perry NS, Houghton PJ, Jenner P, Keith A, Perry EK. *Salvia lavandulaefolia* essential oil inhibits cholinesterase in vivo. Phytomedicine 2002;9(1):48–51.
Rutherford DM, Nielsen MPC, Hansen SK, Witt MR, Bergendorff O, Sterner O. Isolation and identification from *Salvia officinalis* of two Diterpenes which inhibit t-butylbicyclophosphorol [35S]thionate binding to chloride channels of rat cerebrocortical membranes in vitro. Neurosci Lett 1992;135:224–6.
Ryan MF, Byrne O. Plant–insect coevolution and inhibition of acetylcholinesterase. J Chem Ecol 1988;14(10):1965–75.
Savelev S, Okello E, Perry NSL, Wilkins RM, Perry EK. Synergistic and antagonistic interactions of anticholinesterase terpenoids in *Salvia lavandulaefolia* essential oil. Biochem Pharmacol Behav 2003;75:661–8, this issue.
Silva Brum LF, Elisabetsky E, Souza D. Effect of linalool on [2H] MK801 and [^{3}H] muscimol binding in mouse cortical membranes. Phytother Res 2001;15:422–5.
Simpson PM, Wesnes KA, Christmas L. A computerised system for the assessment of drug-induced performance changes in young, elderly or demented populations. Proc BPS 1988;711P–2P.
Sugaya K, Uz T, Kumar V, Manev H. New anti-inflammatory treatment strategy in Alzheimer's disease. Jpn J Pharmacol 2000;82:85–94.
Talesa VN. Acetylcholinesterase in Alzheimer's disease. Mech Ageing Dev 2001;122:1961–9.
Tildesley NTJ, Kennedy DO, Perry EK, Ballard CG, Savelev S, Wesnes KA, Scholey AB. *Salvia lavandulaefolia* (Spanish Sage) enhances memory in healthy young volunteers. Biochem Pharmacol Behav 2003;75:669–74, this issue.
Tinwell H, Lefevre PA, Moffat GJ, Burns A, Odum J, Spurway TD, et al. Confirmation of uterotrophic activity of 3-(4-methylbenzylidine)-camphor in the immature rat. Environ Health Perspect 2002;110(5): 533–6.
Wagner H, Wierer M, Bauer R. In vitro inhibition of prostaglandin biosynthesis by essential oils and phenolic compounds. Planta Med 1986; 184–7.
Wang C-N, Chi C-W, Lin Y-L, Chen C-F, Shiao Y-J. The neuroprotective effects of phytoestrogens on amyloid β protein-induced toxicity are mediated by abrogating the activation of caspase cascade in rat cortical neurones. J Biol Chem 2001;276(7):5287–95.
Wolfe MS. Secretase targets for Alzheimer's disease: identification and therapeutic potential. J Med Chem 2001;44(13):2039–59.
Yamada K, Mimaki Y, Sashida Y. Anticonvulsive effects of inhaling lavender oil vapour. Biol Pharm Bull 1994;17(2):359–60.
Zandi PP, Breitner JCS. Do NSAIDs prevent Alzheimer's disease? And, if so, why? The epidemiological evidence. Neurobiol Aging 2001;22: 811–7.
Zarzuelo A, Risco S, Gamez MJ, Jimenez J, Camara M, Martinez MA. Hypoglycemic action of *Salvia lavandulifolia* Vahl. ssp. oxyodon: a contribution to studies on the mechanism of action. Life Sci 1990;47: 909–15.
Zupko I, Hohmann J, Redei D, Falkay G, Janicsak G, Mathe I. Antioxidant activity of leaves of *Salvia* species in enzyme-dependent and enzyme-independent systems of lipid peroxidation and their phenolic constituents. Planta Med 2001;67:366–8.