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The Psychopharmacology of European Herbs with Cognition-Enhancing Properties

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Abstract: Extensive research suggests that a number of plant-derived chemicals and traditional Oriental herbal remedies possess cognition-enhancing properties. Widely used current treatments for dementia include extracts of *Ginkgo biloba* and several alkaloidal, and therefore toxic, plant-derived cholinergic agents.

Several non-toxic, European herbal species have pan-cultural traditions as treatments for cognitive deficits, including those associated with ageing. To date they have not received research interest commensurate with their potential utility. Particularly promising candidate species include sage (*Salvia lavandulaefolia/officinalis*), Lemon balm (*Melissa officinalis*) and rosemary (*Rosmarinus officinalis*). In the case of sage, extracts possess anti-oxidant, estrogenic, and anti-inflammatory properties, and specifically inhibit butyryl- and acetyl-cholinesterase. Acute administration has also been found to reliably improve mnemonic performance in healthy young and elderly cohorts, whilst a chronic regime has been shown to attenuate cognitive declines in sufferers from Alzheimer's disease.

In the case of *Melissa officinalis*, extracts have, most notably, been shown to bind directly to both nicotinic and muscarinic receptors in human brain tissue. This property has been shown to vary with extraction method and strain. Robust anxiolytic effects have also been demonstrated following acute administration to healthy humans, with mnemonic enhancement restricted to an extract with high cholinergic binding properties. Chronic regimes of aromatherapy and essential oil respectively have also been shown to reduce agitation and attenuate cognitive declines in sufferers from dementia.

Given the side effect profile of prescribed cholinesterase inhibitors, and a current lack of a well tolerated nicotinic receptor agonist, these herbal treatments may well provide effective and well-tolerated treatments for dementia, either alone, in combination, or as an adjunct to conventional treatments.

Key Words: Salvia officinalis, Salvia lavandulaefolia, Melissa officinalis, Rosmarinus officinalis, mood, memory, dementia, cognition.

INTRODUCTION

Several herbal treatments originating from Asia and the Far East are purported to enhance cognitive performance and protect against cognitive decline. These treatments include *Ginkgo biloba* and ginseng (*Panax* species), which are two of most commonly taken herbal treatments throughout the world, and are offered on prescription in a number of countries. A substantial body of research is accumulating into the *in vitro, in vivo* and behavioural modulation effects to support their potential efficacy. It could be argued that this plethora of research results from a scientific curiosity as to the mechanisms and physical effects of tried and trusted herbal treatments that have been in common usage, often for millennia. In line with this, much of the research has been conducted in areas of the world that have long histories of utilisation of the treatments in question, with science in the 'West' only becoming engaged in recent years as the body of research has accumulated, and standardised treatments have become available. Indeed this could also be seen as a consequence of 'Western' society's attachment of 'added value' to synthesised rather than natural medicinal products for many decades. The question that this raises is, in societies that have previously set aside their traditional treatments in favour of synthetic alternatives, which potentially efficacious traditional herbs may have been overlooked.

This question has been addressed by a number of workers in recent years [1-4]. The over-riding rationale underlying the approach is that, whilst traditional European plants have largely been ignored by western medicine in the treatment of dementia, there are a number of plant species reputed, over the centuries or indeed millennia, to have cognition and memory enhancing effects. These plants may well be efficacious in treating the specific biological mechanisms underlying dementia. In the case of Alzheimer's disease these mechanisms may include: disrupted cholinergic transmission; neuronal damage caused by oxidative stress and inflammatory reactions; and beta-amyloid formation or toxicity [3].

With the exception of the NMDA antagonist memantine, current treatments for Alzheimer's disease are restricted to drugs whose principal action is to augment the availability of the neurotransmitter acetylcholine *via* the inhibition of either the cholinesterase (ChE) group of enzymes, or specifically

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acetylcholinesterase (AChE). These treatments are attributed with a '*significant, although modest, effect on the cognitive status of patients with AD*' [5] and have also been shown to improve memory function in young and aged healthy human cohorts [6 and see below].

Perry *et al.* [2] note that a range of cholinergic agents, including both cholinesterase inhibitors used to treat Alzheimer's disease, and muscarinic and nicotinic agonists and antagonists, were initially derived directly from poisonous plants that have no traditional role in memory enhancement (see Table 1).

Furthermore, as alkaloids, these cholinergic agents have a presumed functional provenance as predation deterents, and are all toxic at low concentrations. Thus, they are unsuitable for some dementia sufferers and may require 'step-up' dosing regimens. With this in mind, a pragmatic approach is to investigate the properties of non-toxic European plants traditionally utilised in the enhancement of cognition and memory, with specific reference to their potential to provide non-alkaloid, and therefore less toxic, components capable of interacting with the cholinergic system. To this end a number of *in vitro* investigations of traditionally utilised edible European plants' properties have examined both acetylcholinesterase inhibition [2], and cholinergic receptor interactions in human brain tissue [2, 4] along with potentially beneficial anti-oxidant properties [1].

The results of such investigations suggest that three specific members of the Labiatae family: *Salvia (officinalis* and *lavandulaefolia)* (sage); *Melissa officinalis* (Lemon balm); and *Rosmarinus officinalis* (rosemary) have a long tradition in the treatment of disorders of the central nervous system or memory enhancement. In the case of sage and Lemon balm the have been shown to also possess *in vitro* properties that suggest not only a possible role in the treatment of the cognitive decline associated with dementia, but also in the general cognitive enhancement of healthy populations. The following comprises a review of the history of their usage, evidence of potentially relevant mechanisms and recent demonstrations of potentially beneficial behavioural effects.

SALVIA OFFICINALIS

Historical Perspective

Salvia, with over 700 species, is the largest genus in the Labiatae family. Whilst the genus was recognised and named by both Egyptian and Greek civilizations it owes its name to the Romans (from the Latin salvare - 'to save') [7]. The most common European members of the genus, both originating from the northern shores of the Mediterranean, are *Salvia officinalis* (Garden sage) and *Salvia lavandulaefolia* (Spanish sage).

Perry *et al.* [3] note that medicinal use of members of the genus has developed independently over the millennia in a number of distinct cultures. So, for example, the Greeks considered Garden sage, ('elelisphakon' – *S. officinalis)* to be '*good for helping diminution of senses and loss of memory*' [8], and Ayurvedic medicine (one of the longest surviving systems of herbal medicine), prescribes its use to '*clear emotional obstructions from the mind and for promoting calmness and clarity*' [9]. A number of *Salvia* species have been used in traditional Chinese medicine, with the genus, described as a 'superior' herb, appearing in the *Shen Nung pen tsao* (25BC). Species include *S. plebeia, S. chinensis* and

Table 1. Plant Derived Cholinergic Drugs (Adapted from Perry *et al.* [3])

Type	Chemical	Plant species	Common plant name
Cholinesterase inhibitors	Physostigmine	Physotigma venenosa	Calabar bean
	Galanthamine	*Galanthus nivalis*	Snowdrop
		Narcissus pseudonarcissus	Daffodil
	Huperzine	*Huperzia serrata*	Fern
Muscarinic agonists	Arecoline	*Areca catechu*	Betel nut
	Pilocarpine	*Pilocarpus jaborandi*	
	Muscarine	*Amanita muscaria*	Fly agaric
Muscarinic antagonists	Atropine	*Atropa belladona*	Deadly nightshade
	Hyoscamine	*Hyoscamus niger*	Henbane
	Scopolamine (or hyoscine)	*Mandragora officinarum*	Mandrake
		Datura (numerous species)	e.g. thorn apple
		Scopalia carniolica	
Nicotinic agonists	Nicotine	*Nicotiniana tabacum*	Tobacco
	Lobeline	*Lobelia inflata*	Indian tobacco
	Cytisine	*Laburnum anagyroides*	Laburnum
Nicotinic antagonists	Turbocurarine	*Chondrodendron tomentosum*	
	Sparteine	*Cytisus scoparius*	Broom
	Dihydro-β-erythroidine	*Erythrina* (several species)	
	Methyllycaconitine	*Delphinium brownii*	Delphinium

S. miltiorrhiza (Dan shen), the last of which is reputed to invigorate blood circulation, cool and nourish the blood and calm mental irritability [10]. Interestingly, the use of indigenous varieties of Sage was supplemented with *S. officinalis* following contact with Europeans in both American Indian and Chinese medicine. In the latter case, on its introduction by Dutch traders, *S. officinalis* became preferred above indigenous varieties, with an exchange rate in the Orient of 3:1 finest tea to European sage leaf [11].

The Salerno medical school, the first in medieval Europe, considered locally grown *Salvia fructiosa* to be a 'Herba sacra' (sacred herb). It was described as a cure with a calming effect, and featured in the proverb from the 'Tabuli Salerni', '*Cur moritur, qui salvia crescit in horto*' (Why should he die who has sage in his garden?) [12]. It seems likely that at the same time *S. officinalis* was in common usage throughout Europe, and it is particularly germane to note that it has featured in British herbal apothecaries from the 16th century onwards [13]. Perry *et al.* [3], noting that during this epoch *S. officinalis* was recognised as an enhancer of memory, provide quotations from some of the foremost Herbals of the day [see: 11]. So, for instance; Gerard, in the 16th century, suggests that '*It is singularly good for the head and brain and quickeneth the nerves and memory*'; Culpepper's 'Complete Herbal' notes, in the mid-seventeenth century, that '*It also heals the memory, warming and quickening the senses*'; and John Hill's 'The Family Herbal' tells us, in the 18th century, that, '*Sage will retard the rapid progress of decay that treads upon our heels so fast in latter years of life, will preserve faculty and memory more valuable to the rational mind than life itself*'.

One further curious indication of Sage, which hints at potential anxiolytic effects, is in its traditional use in the mitigation of grief. As an example of this, Pepys notes that "*Between Gosport and Southampton we observed a little churchyard where it was customary to sow all the graves with Sage*" [11].

Contemporary Usage

Specific contemporary indications for *S. officinalis* include: use as a gargle or mouthwash for inflammation of the mouth, tongue or throat; alleviation of flatulent dyspepsia, and loss of appetite; reduction of blood sugar; treatment for cases of respiratory allergy, headache, anxiety and nervousness in the elderly; poor memory, mental confusion, depression, vertigo and as a treatment for the symptoms of the menopause [14-16].

Possible Active Components

In terms of the whole herb, both *S. officinalis* and *S. lavandulaefolia* contain about 1.0 – 2.8 % volatile oil [17]. It has been suggested that the monoterpenoid content of Sage, namely α-pinene, β-pinene, 1, 8-cineole, thujone, camphor and geraniol, contribute to (but may not be completely responsible for) the activity of the whole herb [18]. Extracts also contain a number of polyphenolic compounds, most notably (in order of descending concentration as assessed by densitometry), rosmarinic acid, methyl carnosate, caffeic acid, luteolin 7-0-glucoside, and luteolin [19].

Extracts of *Salvia officinalis* tend to contain alpha- and beta-thujone as a major component (usually around 50%). Whilst *Salvia lavandulaefolia* contains similar components, it lacks the thujone content. Thujone (a terpenoid ketone characterised chemically as bicyclo(3, 1, 0)hexan-3-one, 4-methyl-1-(1-methylethyl)-(1S-(1-, 4, 5-α)) is toxic, and ingestion of large doses causes convulsions [17]. Whilst *S. officinalis* oil does not appear to be as hazardous as its thujone content might suggest [20], it has been suggested that *S. lavandulaefolia* (having negligible thujone), may provide an equally efficacious, but more theoretically suitable, treatment [1].

Potential Mechanisms of Action

Cholinesterase Inhibition

Concentration dependent inhibition of AChE in post-mortem human brain homogenates was demonstrated *in vitro* as a consequence of the application of the essential oils of both *Salvia officinalis* and *S. lavandulaefolia* and alcoholic extracts of both fresh and dried *S. officinalis* leaf [3]. Assessment of the individual activity of the major monoterpenoid constituents (borneol, caffeic acid, camphor, cineol, and thujone) failed to demonstrate inhibition of the enzyme, suggesting another unidentified constituent was responsible. In a similar vein, Perry *et al.* [21] demonstrated dose dependent inhibition of human erythrocyte AChE by *Salvia lavandulaefolia* essential oil, but found that no single constituent was particularly potent, suggesting a synergistic relationship. This *in vitro* anti-cholinesterase activity of the essential oil of *S. lavandulaefolia* has also been confirmed *in vivo* with inhibition of AChE in the striatum and hippocampus of aged rats following oral administration of *S. lavandulaefolia* [22]. This last report is particularly important as it demonstrates that either the active components of the essential oil, or alternatively active metabolites of components of the essential oil, are capable of surviving digestion, and traversing the blood brain barrier for delivery to therapeutically relevant sites.

Given that several terpenoids that have been shown to inhibit AChE occur in the *Salvia* genus [23, 24], and that previous investigations have failed to show substantial AChE inhibition by individual terpenoids in comparison to complete extracts, Savalev *et al.* [25] investigated potential synergistic and antagonistic interactions between constituent terpenoids. Their results suggested that 1, 8-cineole was the most active of the constituents with an IC50 approaching that of the total essential oil (which contained 26.8% 1, 8-cineole). Pairs of terpenoids at naturally occurring ratios demonstrated synergy (1, 8-cineole paired with either α-pinene or caryophyllene) or, alternatively, antagonism (1, 8-cineole and camphor). Mixtures of 6 and 8 terpenoids were less potent than the whole essential oil. Savelev *et al.* concluded that the observed AChE inhibitory activity of the essential oil resulted from a complex interaction of synergistic and antagonistic interactions between the terpenoids, including minor, as yet unidentified, constituents.

Of particular interest here, it should also be noted that the *S. lavandulaefolia* essential oils [26, 27] and an ethanolic *S. officinalis* extract [28] utilised in the acute dose behavioural experiments detailed below, all exhibited significant levels of AChE inhibition in post-mortem human brain tissue.

A single study has also assessed the human erythrocyte butyrylcholinesterase (BuChE) inhibitory properties of *Salvia officinalis*. Savelev *et al.* [29] demonstrated both dose dependent BuChE and AChE inhibition by essential oil. Once again the total inhibition seen as a consequence of the whole oil was not accounted for by the inhibitory properties of the constituents of the oil.

Anti-Oxidant and Anti-Inflammatory Properties

It seems unlikely that anti-oxidant and anti-inflammatory properties underlie demonstrations of acute cognitive enhancement, although it is interesting to note that many cognition enhancing herbal treatments have potent anti-oxidant properties, and the prospect that this is the mechanism underlying their efficacy in this regard has not been tested. However, these properties may well offer long-term protection against the cognitive decline associated with free radical damage and inflammatory reactions.

Oxidative stress plays a role in the general processes of ageing and tissue damage, and is implicated in the pathogenesis of Alzheimer's disease (AD) [30]. Similarly, a role in AD aetiology has been postulated for inflammatory mechanisms [31].

Lamaison *et al.* [32] demonstrated that many species of Labiatae, including *S. officinalis* exhibited free radical (1, 1-diphenyl-2-picrylhydrazyl) scavenging properties.

Both *S. officinalis* and *S. lavandulaefolia* have been shown to have a number of anti-oxidant components [e.g. 33, 34]. In an investigation of the *in vitro* anti-oxidant properties of a number of medicinal plants Mantle *et al.* [35] demonstrated that *S. officinalis* leaf had 'appreciable' levels of anti-oxidant activity (0.32 mmolTE/gm dry weight), of the same order of magnitude as recognised botanical antioxidants such as *Ginkgo biloba* and *Panax ginseng* (0.62 and 0.61 mmolTE/gm dry weight respectively). This result is in line with research by Hohmann *et al.* [19] who demonstrated on the basis both of antioxidant properties and proportional contents of its several polyphenolic components, that the antioxidant properties of a whole extract of *S, officinalis* is probably largely attributable to its rosmarinic acid content. This suggestion is consistent with previous observations of potent radical scavenging activity by this constituent [36, 37]. Net anti-oxidant properties have also been confirmed in an ethanolic extract of *S. lavandulaefolia,* with reduced lipid peroxidation in bovine brain tissue as a consequence of application of the whole extract and five out of six monterpenoids (α and β-pinene, geraniol, 1, 8-cineole, and thujone). The sixth - camphor (the largest constituent of an essential oil assessed by gas-chromatography in the same study) was shown to have pro-oxidant properties [18].

Support for a traditional role for *Salvia* species as anti-inflammatories [15] was offered by the demonstration of anti-inflammatory actions (eicosanoid inhibition in rat leucocytes) by an ethanolic extract of *S. lavandulaefolia* and its geraniol and α-pinene monoterpenoid constituents [18].

Oestrogenic Properties

Several studies indicate that oestrogen replacement therapy is associated with both a lowered risk of Alzheimer's disease, and amelioration of the symptoms of Alzheimer's disease [38], although the evidence is not unequivocal in this respect [e.g. 39, 40]. Oestrogen receptor stimulation can produce a number of pharmacological effects [41], including increased cerebral blood flow, anti-inflammatory actions, enhancement of synaptic activity, and neuro-protective and neuro-trophic effects on brain tissue [42]. Contemporary uses of sage include its use as a component of preparations for the treatment of gynaecological problems [15] and, in the UK, as an over the counter treatment for the relief of symptomatic disturbances associated with the menopause (e.g. Holland and Barrett dried sage leaf capsules). This role receives cautious support from an open trial showing the amelioration of menopausal symptoms in 30 women by a product containing extracts of *S. officinalis* and *Medicago sativa* (alfalfa) [43]. In line with suggestions that members of the *Salvia* genus may have oestrogenic properties[3], Perry *et al.* [18] demonstrated weak but significant estrogenic activity in the essential oil of *S. lavandulaefolia*, and the monoterpenoid component geraniol, when applied to a recombinant yeast screen expressing genomically integrated human estrogen receptors.

Acute Behavioural Studies

The behavioural effects of acute doses of the *S. lavandulaefolia* and *S. officinalis* have been assessed in several double-blind, placebo-controlled, randomised, balanced-crossover studies in healthy humans. In the first of these Tildesley *et al.* [26] report the results of two experiments investigating the acetylcholinesterase inhibiting properties of commercial *Salvia lavandulaefolia* essential oils (average IC_{50} = 0.07mg/ml in bovine enzymes), and their subsequent effect on immediate and delayed recall of word lists. In the first experiment the mnemonic effects of 50, 100 and 150 µl of encapsulated essential oil in 20 healthy young volunteers, 1 hr, 2.5 hrs, 4 hrs and 6 hrs after administration were assessed. The results showed significant improvements in both immediate and delayed word recall for the lowest dose (50 µl) at the first two post-dose testing sessions (1 and 2.5 hrs), and for the middle (100 µl) dose at the 2.5 hour testing session. As these results suggested that the lowest dose was the most effective, the second experiment investigated the possibility that an even lower dose may be more beneficial, and compared the effects of 25 and 50 µl in 24 young volunteers. The 50 µl dose proved most effective again, with significant improvements seen in immediate and delayed word recall at 1 and 4 hrs post-dose. Whilst failing to reach significance the 25 µl dose evinced trends towards improved immediate ($p=0.051$) and delayed ($p=0.073$) word recall at 1 hr post-dose.

In a second report, Tildesley *et al.* [27] examined the effects of 25 and 50 µl of *S. lavandulaefolia* oil on mood and performance of the Cognitive Drug Research computerised assessment battery and serial subtraction tasks. Both doses of sage were associated with improved performance on memory factors derived from the individual CDR battery outcomes, with 25 µl improving scores on both a 'Secondary Memory' (accuracy across 4 memory tasks) factor at 1 hr post-dose and a 'Speed of Memory' factor (speed of performing 4 memory tasks) at 2.5 hrs. The higher dose (50 µl) led to improved 'Speed of Memory' at the later time points (4 and 6 hrs). Both doses also resulted in faster performance on the

Serial Sevens subtraction task, but increased errors on the Serial Threes task. However, perhaps the most striking finding from this study was of beneficial modulation of mood across the testing sessions, with significantly increased ratings of 'alertness', 'contentedness' and 'calmness' for the 50 µl dose and increased ratings on the latter two measures for the 25 µl dose.

These two initial studies had been undertaken utilising *S. lavandulaefolia* in healthy young participants. Therefore, a further study [28] assessed the effects of four separate single doses (167, 333, 667, 1332 mg) of an ethanolic extract of *Salvia officinalis* with AChE inhibitory properties in an elderly cohort (n = 20, mean age 72.9 years) with presumed, natural, age-related down-regulation of cholinergic integrity [44]. The study used the same methodology and cognitive/mood assessment as the previous study [27]. The results again showed clear improvements in memory performance, in this case largely restricted to the two lower doses, with the 167 mg dose evincing improved 'Secondary Memory' performance at 2.5 and 4 hrs post-dose, whilst the 333 mg dose led to significantly improved 'Secondary Memory' at all post-dose time points, with concomitant improvements on the 'Accuracy of Attention' factor (accuracy of performing three attention tasks). While these effects may be attributed to the extracts' pro-cholinergic properties, other mechanisms outlined above may also be involved.

Chronic Administration in Alzheimer's Disease

To date two studies have also addressed the effects of sage in cohorts suffering from mild to moderate probable Alzheimer's disease. Perry *et al.* [45] conducted a preliminary, open label clinical trial, in 11 participants, primarily assessing the tolerability of 6 weeks oral administration (50 µl twice per day) of an AChE inhibitory (IC50 = 0.116 µl/ml) *S. lavandulaefolia* essential oil, but with several measures of cognitive performance and psychopathology included. Although the trial did not include a placebo condition the results were promising, with excellent tolerability of the extract, and suggestive significant improvements at the 6 week end-point seen on both the accuracy of performing a vigilance task and in dementia psychopathology as assessed by the Neuropsychiatric Inventory (NPI), both in comparison to the outset of the trial.

A double-blind, placebo-controlled trial assessing the effects of 16 weeks administration of a *Salvia officinalis* alcoholic tincture in 39 patients (placebo, n = 20 / *S. officinalis*, n=19) has also been reported [46]. Whilst both placebo and *S. officinalis* groups were reduced to 15 by drop-outs, those in the *S. officinalis* group were shown to have significantly improved scores on the Alzheimer's Disease Clinical Assessment Scale cognitive subscale (ADAS-cog) in comparison to placebo at the study endpoint at 16 weeks. Similarly, clinical ratings (Clinical Dementia Rating) were significantly improved at the end of the study. However, these results require a note of qualification. The extract (60 drops of a 1:1 alcohol:dried leaf tincture per day) was ill defined and no description of the placebo was provided. Furthermore, the pattern of results, with a substantial decline on the ADAS-cog (approximately 7 points over 16 weeks) for the placebo group and a large concomitant rise (approximately 5 points) for the treated group, would not normally be expected in a clinical trial of this duration and nature. To give an example, Le Bars *et al.* [47] conducted a double-blind, placebo-controlled trial of *Ginkgo biloba* extract in a large patient group (N = 236) suffering mild to severe Alzheimer's. They reported a mean decline in ADAS-cog scores of a mere 1.5 points by the 52 week end-point for the placebo group, with a total significant difference of 1.7 points between treatment groups. Whilst the results from Akhondzadeh *et al*s' trial are promising, it seems most parsimonious to suggest that the magnitude of the treatment effect and decline in ADAS-cog scores for placebo may represent either a product of the low power of the trial (N = 30), or alternatively an interaction with some other factor.

That being the case, this trial does still argue for a concerted effort to establish the efficacy of sage in treating groups suffering a pathological down-regulation of cholinergic function.

MELISSA OFFICINALIS

Historical Perspective

Melissa officinalis (Lemon balm), is a cultivated perennial lemon scented herb. Originating in Southern Europe its cultivation and use spread throughout Europe by the Middle ages.

Records concerning the medicinal use of *M. officinalis* date back over 2000 years with entries in the 'Historia Plantarum' (approximately 300 BC) and the 'Materia Medica' (approximately 50-80 BC). Medicinal use throughout this early epoch include a recommendation by Paracelsus (1493-1541) that balm would completely revivify a man, and indication for "all complaints supposed to proceed from a disordered state of the nervous system" (see: Grieve, 1931). Several early herbal Apothecaries also attributed balm tea not only with general beneficial effects upon the brain, but also with specific mnemonic improvements. These include, from the 17th century, John Evelyn who writes that "Balm is sovereign for the brain, strengthening the memory and powerfully chasing away melancholy"; and the authors of 'The London Dispensary' who recommend Balm for the 'strengthening of the brain'.

Contemporary Usage

Approximately 50 tons of balm leaves are sold each year for medicinal purposes in Germany alone, much of which is cultivated in Eastern European countries and Spain. Contemporary reports suggest that, as well as possessing spasmolytic and antibacterial properties, *Melissa officinalis* can modulate a number of behavioural measures, with indications including administration as a mild sedative, in disturbed sleep, and in the attenuation of the symptoms of nervous disorders, including the reduction of excitability, anxiety, and stress [49, 50]. In keeping with its long history of safe usage no adverse side effects have so far been reported for the herb [51]. *Melissa officinalis* is predominantly sold 'over the counter' as a popular herbal food supplement, most often is combined with other herbs. As an illustration of this, the German pharmaceutical industry's 'Rote Liste' (2001) drug catalogue included 49 products containing lemon balm.

Possible Active Components

A number of possible active components of the dried leaf and essential oil of the herb have been identified. Constituents that may have pharmacological effects include a number of monoterpenoid aldehydes (including citronellal, neral and geranial), [52, 53], flavonoids and polyphenolic compounds (most notably rosmarinic acid) [52, 54] and monoterpene glycosides [55].

Variations in the percentage content of individual components in balm oil can be attributable to a number of factors. For instance, varying origins and harvest times, the number of years of cultivation, developmental stage of the specific harvested leaf, the use of fresh or dried material, the nature of the drying and oil extraction process, and the duration of the storage of the plant product [56].

Potential Mechanisms of Action

Acetylcholinesterase Inhibition

Two commercial *M. officinalis* oils were found to have substantial acetylcholinesterase inhibiting properties in human brain homogenates [2]. However, this finding has to be treated with caution as lime oil, which is often an added ingredient in commercial balm preparations, exhibited similar properties. Fresh leaf, but not dried leaf, also exhibited modest anti-cholinesterase activity with a % inhibition (26.4 % inhibition at 2 mg leaf/ml) of something over half that exhibited by *Salvia officinalis* fresh leaf (47% inhibition at 2 mg leaf/ml).

Cholinergic Receptor Binding Properties

Extracts of dried *M. officinalis* leaf have been shown to exhibit central nervous system acetylcholine receptor activity, with demonstrations of both nicotinic [2, 4] and muscarinic [4] binding properties in homogenates of human brain tissue. In the case of the latter study, six separate accessions of *M. officinalis* leaf elicited markedly different proportions of binding to the two acetylcholine receptor subtypes in human occipital cortex tissue, with IC50 concentrations ranging from 0.08 mg to 3.8 mg/ml for the displacement of [3H]-(N)-nicotine from nicotinic receptors, and from 0.5 to >5 mg/ml for the displacement of [3H]-(N)-scopolamine from muscarinic receptors. The cholinergic receptor binding properties of a commercial methanolic extract [57] and eight samples of organic dried leaf [58] have also been assessed in post-mortem brain tissue. In the case of the former study [57] negligible nicotinic binding and comparatively low muscarinic binding were demonstrated, with this absence putatively attributed to the manufacturing process. However, in the case of the latter study [58] whole extracts of all eight samples exhibited substantial but varying nicotinic (IC50s ranging from 0.18 to 3.16 mg/ml) and muscarinic (IC50s ranging from 1.46 to 4.31 mg/ml) receptor binding properties.

Anti-Oxidant and Anti-Inflammatory Properties

It has been demonstrated that many species of Labiatae, including *M. officinalis* exhibit free radical (1, 1-diphenyl-2-picrylhydrazyl) scavenging properties [59, 60].

In an investigation of the *in vitro* anti-oxidant properties of a number of medicinal plants Mantle *et al.* [35] demonstrated that *Melissa officinalis* leaf had modest but 'appreciable' levels of anti-oxidant activity (0.18 mmolTE/gm dry weight), in comparison to recognised antioxidants such as *Ginkgo biloba* and *Panax ginseng* (see above). This result is in line with research by Hohmann *et al.* [54] who demonstrated that, as with *S. officinalis*, on the basis both of inhibiton of enzyme dependent, and independent, lipid peroxidation and the proportional contents of its several phenolic components, the antioxidant properties of a whole extract of *M. officinalis* is probably mainly attributable to its flavonoid content.

Animal Studies

In line with its contemporary role as a mild sedative, a number of studies involving rodents suggest specific 'calming' or sedative effects. Examples include a reduction in spontaneous movement demonstrated in mice as a consequence of both the whole volatile oil of melissa and the individual isolated terpenes, with prolongation of hexobarbital induced sleep in mice after even the lowest dose (1mg/kg) of oil administered orally [61]. Similarly, reductions in behavioural parameters in mice on both familiar and non-familiar environment tests were elicited by an hydro-alchoholic extract of *M. officinalis*, but not by essential oil [62]. An inverted U shaped dose response was evident with the greatest effect following 25 mg/kg (dose range 6-100 mg/kg). The plant extract also increased pentobarbital induced sleep parameters. This effect was also later observed to follow an inverted U dose response pattern with the soporific effect increasing up to 15 mg/kg then decreasing at doses between 200 and 400 mg/kg [62].

Human Behavioural Studies

Two studies have investigated the effects of a valerian/melissa combination on sleep quality. In the first, similar improvements were demonstrated as those associated with 0.125 mg of the benzodiazepine Triazolam in poor sleepers, but with no adverse 'rebound' effects [63]. Similarly, significant improvements in quality of sleep were demonstrated for 66 healthy participants in a 360 mg valerian/240mg melissa condition, in comparison to a placebo group, over a 30 day dosing period [64].

A series of double-blind, placebo-controlled, balanced-crossover studies have also assessed the behavioural effects of single doses of *M. officinalis* using a similar methodology to that employed in the investigations of *S. officinalis* (see above). In the case of the first two studies [57, 58] concomitant investigations both of *in vitro* cholinergic receptor binding in human post-mortem brain tissue and modulation of cognitive performance and mood following oral administration of *M. officinalis* were undertaken. In the first of these [57], three separate single doses of a commercial methanolic *M. officinalis* extract were compared to placebo. Twenty participants received the four separate treatments (placebo, 300mg, 600mg, and 900mg of a commercial methanolic M. officinalis extract) at weekly intervals, in an order dictated by random allocation to a Latin square. Assessment of mood and cognitive performance took place pre-

dose and at 1 hour, 2.5 hours, 4 hours and 6 hours post-dose. The most notable result of this experiment was a striking dose-dependent impairment on a 'Quality of Memory' measure derived by factor analysis from the Cognitive Drug Research (CDR) computerised assessment battery. More specifically, decrements were most pronounced, and increased with dose, on two timed memory tasks (delayed word recognition and a spatial memory task). The overall pattern of results also showed that the lowest dose (300 mg) engendered increased 'calmness' at the first two post-dose testing sessions (1 hour and 2.5 hours), and the middle dose (600 mg) led to improved performance on attention tasks. The highest dose, however, was not associated with any benefits, and led to the most pronounced decrements on the memory tasks along with reduced 'alertness' at all post-dose testing sessions. Whilst this pattern of results is broadly in line with the contemporary role of *M. officinalis* as a calming agent and mild sedative, it is not in keeping with beneficial modulation of cholinergic activity. Indeed, the *in vitro* analysis of the extract (completed after the behavioural experiment) showed that the extract in question showed negligible displacement of [^{3}H]-(N)-nicotine from nicotinic receptors, and comparatively low displacement of [^{3}H]-(N)-scopolamine from muscarinic receptors in human brain tissue.

As this left open the question of the cognitive and mood effects of a cholinergically 'active' *M. officinalis* the second experiment [58] was conducted in two distinct phases. In the first phase an initial *in vitro* investigation of the cholinesterase inhibitory and cholinergic receptor binding properties of eight acquisitions of organically grown *M. officinalis* of known provenance was undertaken. Whilst none of the samples of dried leaf led to measurable inhibition of cholinesterase, all eight samples showed a substantial affinity for muscarinic receptors. The sample chosen for the behavioural experiment was one of a number of samples that also exhibited a substantial affinity for nicotinic receptors (IC^{50} concentrations of 0.18 mg ml-1 and 3.47 mg ml-1 respectively for the displacement of [^{3}H]-(N)-nicotine and [^{3}H]-(N)- scopolamine). The second phase of the experiment utilised a similar methodology to the previous study, with participants receiving three single doses of the dried M. officinalis leaf (600mg, 1000mg, 1600mg) and a placebo, counterbalanced, at seven day intervals. Testing took place pre-dose and at 1, 3 and 6 hours post-dose using the CDR battery, in this case augmented with a 5 minute 'cholinergically sensitive' rapid visual information processing task (RVIP). The results showed that whilst the lowest dose was associated with decrements on both the RVIP and the same timed memory tasks as had been disrupted in the previous study, these effects attenuated with increasing dose. The highest (1600 mg) dose of dried leaf led not only to increased 'calmness' (as measured by Bond-Lader mood scales) at all post-dose time points, but also significantly improved performance on the 'Quality of Memory' factor at the 3 and 6 hours post-dose testing sessions.

The pattern of results from the two studies was speculatively interpreted as reflecting the workings of more than one mechanism [58]. It seems likely that the decrements on the more difficult timed tasks - which were seen to decrease with increasing doses of dried leaf, and increase with dose following ingestion of the more highly concentrated manufactured extract - reflect the working of a single mechanism. Furthermore, given that the manufactured extract had little cholinergic activity, it would seem that one or more different mechanisms were responsible for both these effects and the improved calmness seen following the most beneficial dose of each treatment. The remaining memory improvements seen for the highest dose of the 'cholinergic' *M. officinalis* leaf could certainly be accommodated within the expected pattern of 'cholinergic' effects.

In partial support of this interpretation (at least with respect to mood effects), a further study examined the effects of the methanolic *M. officinalis* extract on laboratory induced acute psychological stress [65]. This double-blind, placebo-controlled, counterbalanced cross-over study assessed the mood and performance effects of two separate doses (300 mg, 600 mg) of the methanolic commercial extract of *M. officinalis* during the performance of the 'Defined Intensity Stress Simulator' (DISS) laboratory stressor battery by 18 healthy young participants. Whilst completion of the battery itself led to increased subjective ratings of alertness and reduced ratings of calmness, the 600 mg dose of *M. officinalis* extract led to a direct significant attenuation of these negative mood effects.

Chronic Administration in Alzheimer's Disease

Two recent double-blind, placebo-controlled studies have also assessed the effects of M. officinalis in sufferers from dementia. Ballard *et al.* [66] examined the effect of *M. officinalis* essential oil aromatherapy (in comparison to vegetable oil) on ratings of agitation and quality of life of 71 patients suffering from severe dementia. Following 4 weeks treatment patients in the active treatment group were rated, in comparison to the placebo group, as being less agitated, less socially withdrawn, and as engaging in more time spent in constructive activities.

Utilising a similar methodology as their study into the effects of *S. officinalis* (see above), Akhondzadeh *et al.* [67] also assessed the effects of 60 drops/day of a tincture of *M. officinalis* in the 35 sufferers from mild to moderate dementia (20 verum, 15 placebo) that completed their 16 week trial. At the study end-point the results showed a clear cognitive advantage (ADAS-cog and Clinical Dementia Rating) and reduced agitation for the *M. officinalis* group. Once again, however, the profile of the declines for the placebo group (+5.6 points on the ADAS-cog) and the improvements for the *M. officinalis* group (-6.4 points) would not have been anticipated from the results of larger trials assessing the effects of other anti-dementia treatments.

ROSEMARINUS OFFICINALIS

Historical Perspective

Rosemary has a long and eminent history as a sacred herb, associated particularly with the remembrance of love and death. This history originates with the ancient Egyptians' use of the herb in the mummification process, and spans the cultures of ancient Greece and the Romans. In these latter societies one of its other, more curious uses was as a memory enhancer [68]. Greek students wore sprigs or garlands of rosemary at times of educational demand, and Roman stu-

dents massaged their temples and foreheads with rosemary oil prior to examinations. This notion of rosemary as a cognition enhancer survives to the present day, with references sprinkled throughout the historical record. Possibly one of the most famous instances is Shakespeare's "There's rosemary; that's for remembrance" (Ophelia in 'Hamlet'), but reference to the mnemonic potential of Rosemary can also be found in the writings of most herbal apothecaries from the 15th century onwards. Examples from the 16th and 17th centuries include allusions by Culpepper ("*It helps a weak memory, and quickens the senses*"), and John Gerard ("*Rosemary comforteth the braine, the memorie, the inward senses*") and indication for "*weyknesse of the brain*" in the Grete Herball.

Contemporary Usage

Herbal medicinal indications include internally as a tonic, stimulant, and carminative to treat flatulent dyspepsia, stomach pains, headaches, and nervous tension, and as a treatment for memory problems [15-17]. Its external use is also recommended as a mild antiseptic, in the promotion of wound healing, and in rheumatic and peripheral circulatory disorders [69].

Potential Mechanisms of Action and Active Components

Little evidence is yet available with regards mechanisms that are potentially relevant to cognitive function. The most widespread uses of rosemary are as an anti-microbial [70] and anti-oxidant [71] agent in foodstuffs, and as a culinary flavouring.

The role as an anti-oxidant has received the most attention, and the various active components of rosemary extracts have all been attributed with substantial anti-oxidant properties. These include several phenolic diterpenes, most notably carnosol and carnosic acid [72, 73], flavonoid glycosides including luteolin [74, 75] and the phenolic compounds, rosmarinic acid and caffeic acid [76].

Extracts also contain a number of potentially active components in common with the other Labiatae, including 1, 8-cineole, a-pinene, camphor, and borneol [17, 50].

Given that *Rosmarinus officinalis* was identified as one of three European medicinal plants with a consistent history of usage as a cognition enhancer, Perry *et al.* [2] included a fresh leaf and an essential oil in their assessment of cholinergic receptor binding and acetylcholinesterase inhibition in human brain tissue. In both assays Rosemary oil showed measurable activity, but at levels of receptor binding substantially below that exhibited by *Melissa officinalis* and at levels of acetylcholinesterase inhibition markedly below *Salvia officinalis* leaf and oil. Given the need to focus research priorities on plants with the most compelling *in vitro* properties, further *in vitro* and human behavioural investigations concentrated solely on *M. officinalis* and *S. officinalis*.

Human Investigations

Two studies have, however, assessed the effects of rosemary (and lavender) essential oil aromatherapy in humans.

Diego *et al.* [77] assessed the Electroencephalograph (EEG) effects of 3 minutes aromatherapy in 40 participants. Following Rosemary they reported decreased frontal alpha and beta power, which they interpreted as suggesting increased alertness. They also found participants in the rosemary condition to report reduced state anxiety and perform mathematics computations faster.

In a recent single-blind study, Moss *et al.* [78] also assessed the effects of lavender and rosemary essential oils and no scent on performance of the CDR computerised assessment battery and mood in 140 participants. They found that those in the rosemary aromatherapy condition exhibited a significant enhancement of memory task performance, but with a corresponding significant reduction in the speed of memory task performance. Subjective ratings of alertness and contentedness were also increased in comparison to the no-scent condition.

CONCLUSIONS

The converging lines of historical, mechanistic and behavioural evidence suggest that both sage (*S. officinalis/lavandulaefolia*) and Lemon balm (*M. officinalis*) extracts may enhance cognitive performance in healthy cohorts and provide a well-tolerated treatment for age and disease associated declines in cognitive function. In particular, the AChE inhibitory properties of sage extracts, and cholinergic receptor interactions of *M. officinalis*, offer potential treatments for the cholinergic down-regulation seen in dementia, without the negative side effect profiles associated with 'alkaloid' cholinergic agents. In the case of sage, the results of the acute behavioural studies, particularly the robust enhancement of memory performance [26-28] and tasks requiring attentional resources [27, 28], are largely consistent both with the anticipated effects of increased availability of acetylcholine [e.g. 44], and previous demonstrations of modulation of cognitive performance in healthy cohorts by cholinesterase inhibitors [6]. With regards *M. officinalis*, improved cognitive functioning in sufferers from Alzheimer's disease [67] and improved memory in healthy cohorts following a 'nicotinic/muscarinic' dried leaf [58] are supportive of the notion that enhancement is due to cholinergic modulation. Whilst this is encouraging, it remains possible that mechanisms other than AChE inhibition and cholinergic receptor binding properties (respectively) are involved, and that different strains and extractions of members of the Labiatae genera will have differing profiles of relevant properties, and thus behavioural effects. As a concrete example of this, variability in nicotinic and muscarinic receptor binding in human brain homogenates varied considerably across strains of *Melissa officinalis* [4]. An extract with negligible cholinergic receptor binding produced behavioural results consistent with its long traditional use as a mild sedative/anxyolitic but did not enhance memory [57], whereas an extract specifically chosen for its high muscarinic and nicotinic binding properties in human brain tissue had the same mood effects but also improved memory performance [58].

This last example also suggests that, in the case of *M. officinalis*, the robust calming/anxyolitic effects of the plant [57, 58, 65, 66] are dependent on an, as yet unidentified, non-cholinergic mechanism. This does highlight the need for a thorough investigation of a wide range of properties for each species. Where AChE (and more latterly BuChE) inhi-

bition and cholinergic receptor binding have been comparatively well researched in sage and *M. officinalis* respectively, the converse cannot be said. Nor have a host of other mechanisms potentially relevant to cognitive enhancement been adequately investigated. In the case of cholinergic receptor binding alone, whilst gross muscarinic and nicotinic binding have been demonstrated, it is unclear whether this is taking place at 'cognition relevant' receptor sub-types, such as the muscarinic M1 [79, 80], M2 [81] and M4 [82] receptors, and nicotinic $\alpha4\beta2$ and $\alpha7$ receptors [83]. Similarly, the mood/anxiolytic effects, and potentially some aspects of the pattern of cognitive modulation, may be attributable to interactions with $GABA^A$ receptors [84, 85] and this possibility deserves some attention.

In the case of Rosemary, investigation of its potentially relevant properties (with the exception of anti-oxidant potential) stalled at the demonstration of only weak AChE inhibition and cholinergic receptor binding by a single essential oil [2]. Subsequently, and as a consequence of a pragmatic focussing of research efforts, it has not benefited from further *in vitro* investigation or human behavioural studies. Given that this herb has an equally illustrious history as a cognition enhancer it might usefully benefit from further research efforts.

Synthesis or Synergy

A fuller understanding of the active constituents of these Labiatae, and their physiological/behavioural effects, will necessarily raise the question as to whether individual components should be isolated or synthesised. Whilst this has been the case with the majority of other plant based treatments, the possibility exists that the benefits of the combined components of the whole plant are greater than the sum of the individual components.

This is most evident where studies (in this case, of *S. officinalis*) have assessed both whole extracts or essential oils and the main putatively active constituents for antioxidant [16], anti-inflammatory [18] and AChE [2, 21, 25] and BuChE [29] inhibitory properties. The results of these studies have invariably suggested that the properties of the whole extract/oil are more potent than the sum of the constituent parts. Whilst a failure to identify and/or isolate potentially potent minor constituents may account for this, it is also possible that the gross effects of whole extracts represent a synergistic relationship between the constituent parts. Only one study has directly addressed this possibility [25- see above]. The authors conclude that both synergistic and antagonistic AChE inhibitory relationships occur between selected constituents at normally occurring ratios, with greater activity for the essential oil than the constituents. It appears that the ChEI inhibitory properties of the oil represent complex agonistic and antagonistic synergistic interactions.

The concentrations of the active components in herbal extracts and preparations vary with the specific extraction method. Similarly, the levels of active components in plant material depend on growth conditions, such as climate, soil composition, light levels and time of harvest [86]. As an example, levels of the potentially toxic cis-thujone and of the pro-oxidant camphor in sage essential oils have been shown to be at their lowest in April in plants grown in widely separated geographical locations [86, 87], whilst wide differences in % concentrations of 1, 8-cineole and camphor have been demonstrated in samples of the same species cultivated in differing geographic locations [88]. Contemporary agricultural techniques, including highly controlled hydro-ponic environments, now offer the opportunity to tightly control the growing environment, including the introduction of stressors that provoke specific changes in levels of active components. It now seems feasible to grow well-standardised plants that benefit from augmented and constrained levels of beneficial and detrimental components respectively, while retaining the positive synergistic properties of whole extracts. As an example, Savelev *et al.* [25, 29] on the basis of their investigations of synergy within the chemical constituents of *Salvia lavandulaefolia*, suggest that plant material with high 1, 8-cineole and low camphor content would be the optimum for AChE inhibition.

Whether the potential benefits of highly controlled natural extracts over isolated and synthesised constituents survives the commercial/patent imperatives of the pharmaceutical sector remains to be seen. However, the possibility of tailor made natural extracts rich in the most advantageous constituents requires a much fuller understanding of the interaction and synergy between active components, physiological activity and CNS and behavioural modification.

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