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Modulation of mood and cognitive performance following acute administration of *Melissa officinalis* (lemon balm)

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Abstract

Melissa officinalis (lemon balm) is a traditional herbal medicine, which enjoys contemporary usage as a mild sedative, spasmolytic and antibacterial agent. It has been suggested, in light of in vitro cholinergic binding properties, that *Melissa* extracts may effectively ameliorate the cognitive deficits associated with Alzheimer's disease. To date, no study has investigated the effects on cognition and mood of administration of *Melissa* to healthy humans. The present randomised, placebo-controlled, double-blind, balanced-crossover study investigated the acute effects on cognition and mood of a standardised extract of *M. officinalis*. Twenty healthy, young participants received single doses of 300, 600 and 900 mg of *M. officinalis* (Pharmaton) or a matching placebo at 7-day intervals. Cognitive performance was assessed using the Cognitive Drug Research (CDR) computerised test battery and two serial subtraction tasks immediately prior to dosing and at 1, 2.5, 4 and 6 h thereafter. In vitro IC_{50} concentrations for the displacement of [^{3}H]-(*N*)-nicotine and [^{3}H]-(*N*)-scopolamine from nicotinic and muscarinic receptors in human occipital cortex tissue were also calculated. Results, utilising the cognitive factors previously derived from the CDR battery, included a sustained improvement in Accuracy of Attention following 600 mg of *Melissa* and time- and dose-specific reductions in both Secondary Memory and Working Memory factors. Self-rated "calmness," as assessed by Bond–Lader mood scales, was elevated at the earliest time points by the lowest dose, whilst "alertness" was significantly reduced at all time points following the highest dose. Both nicotinic and muscarinic binding were found to be low in comparison to the levels found in previous studies.

Keywords: Acute effects; Attention; Cholinergic; *Melissa officinalis*; Memory; Mood

1. Introduction

Melissa officinalis (lemon balm) is a cultivated perennial lemon scented herb. Records concerning its use date back over 2000 years with entries in the *Historia Plantarum* (approximately 300 B.C.) and the *Materia Medica* (approximately 50–80 B.C.). From its Moorish introduction into Spain in the seventh century, its cultivation and use spread throughout Europe by the middle ages (Koch-Heitzmann and Schultze, 1988). 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" (Grieve, 1980). Several herbal apothecaries of the time also attributed balm tea not only with general beneficial effects upon the brain but also with specific mnemonic improvements (Coghan, 1584; Evelyn, 1699).

Contemporary reports stress the sedative, spasmolytic and antibacterial effects of ingestion of *M. officinalis*, with indications encompassing nervous disorders including the reduction of excitability, anxiety and stress, gastrointestinal disorders and sleep disturbance (Kommission E Monograph, 1984; Bisset and Wichtl, 1994). In keeping with its long history of safe usage, no side effects have so far been reported (Wong et al., 1998).

M. officinalis is predominantly sold in combination with other herbs, with, as an illustration, 49 products containing lemon balm in the German pharmaceutical industry's current "Rote Liste" (2001) drug catalogue.

A number of studies involving rodents suggest specific "calming" or sedative effects. Examples include a reduction

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in spontaneous movement demonstrated in mice as a consequence of both the whole volatile oil of *Melissa* and the individual isolated terpenes (Wagner and Sprinkmeyer, 1973). Similarly, reductions in behavioural parameters in mice on both familiar and nonfamiliar environment tests were elicited by a hydroalcoholic extract of *Melissa*. 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 (Soulimani et al., 1991).

Whilst no studies have looked at the effects on humans of the ingestion of *Melissa* by itself, several have investigated the effects of a valerian/*Melissa* combination on sleep quality, with, for example, similar improvements demonstrated as those associated with 0.125 mg of triazolam in poor sleepers (Dressing et al., 1992) and significant improvements in quality of sleep, in comparison to placebo, during 30 days of treatment with 600 mg/day of a combination including the *M. officinalis* extract utilised in the current study (Cerny and Schmid, 1999).

A single, recent, double-blind, placebo-controlled study (Ballard et al., in press) also examined the effect of *M. officinalis* essential oil aromatherapy on ratings of agitation and quality of life of 71 patients suffering from severe dementia. Following 4 weeks of treatment, patients in the active treatment group were rated, in comparison to the placebo group, as less agitated, less socially withdrawn and as engaged in more time spent in constructive activities.

Behavioural consequences such as these could be attributable to a number of possible active components of the dried leaf and essential oil of the herb. Constituents that have been identified include a number of monoterpenoid aldehydes (including citronellal, neral and geranial) (Carnat et al., 1998), flavonoids and polyphenolic compounds (most notably rosmarinic acid) (Carnat et al., 1998; Hohmann et al., 1999) and monoterpene glycosides (Mulkens et al., 1985).

It has been suggested, on the basis of a retrospective review of the historical role of a number of European plant species in the enhancement of memory, that *Melissa* and another plant in the Labiatae family, *Salvia officinalis* (Sage), might potentially provide novel natural treatments for Alzheimer's disease (Perry et al., 1999). This approach has generated research showing that *M. officinalis* exhibits central nervous system (CNS) acetylcholine receptor activity, with demonstrations of both nicotinic (Perry et al., 1996; Wake et al., 2000) and muscarinic (Wake et al., 2000) binding properties. In the case of the latter study, six separate accessions of *Melissa* leaf elicited markedly different proportions of binding to the two acetylcholine receptor subtypes in human occipital cortex tissue, with IC_{50} concentrations ranging from 0.08 to 3.8 mg/ml for the displacement of [^{3}H]-(*N*)-nicotine from nicotinic receptors and from 0.5 to >5 mg/ml for the displacement of [^{3}H]-(*N*)-scopolamine from muscarinic receptors. These properties might provide a potential treatment for the cholinergic disturbances in Alzheimer's disease. Additionally, demonstrations of antioxidant activity (Hohmann et al., 1999; Mantle et al., 2000) suggest that *Melissa* may also provide some protection against the putative aetiological free radical damage in dementia.

Given its long history as a putative memory enhancer, contemporary usage as a mild sedative, sparse but suggestive animal studies and the recent delineation of possible specific CNS neurotransmitter effects, it was considered important to investigate the cognitive effects of administration of *M. officinalis* to humans.

The Cognitive Drug Research (CDR) integrated computerised test battery has previously been shown to be sensitive to the cognitive effects of both acute and chronic administration of *Ginkgo biloba* (Kennedy et al., 2000; Wesnes et al., 1987), acute administration of ginseng (Kennedy et al., 2001a) and both acute doses of a *G. biloba*/*Panax ginseng* combination administered to healthy young volunteers (Kennedy et al., 2001b) and a chronic regimen in healthy neurasthenic and middle-aged cohorts (Wesnes et al., 1997, 2000).

The present study investigated the dose–response relationship and time course of possible changes in mood and cognitive performance in healthy young volunteers following single doses of *M. officinalis*, with reference primarily to the global cognitive domain factors (Speed of Attention, Accuracy of Attention, Quality of Memory and Speed of Memory) and memory subfactors (''secondary'' and ''working'' memory) that can be derived from the complete CDR battery (Wesnes et al., 1999, 2000). Other measures included single task outcomes from the CDR battery, computerised ''serial subtraction'' mental arithmetic tasks (Scholey et al., 2001; Scholey and Kennedy, 2002) and Bond–Lader Visual Analogue Mood Scales (Bond and Lader, 1974). Nicotinic and muscarinic binding properties for the specific *M. officinalis* extract were investigated using the in vitro methods utilised by Wake et al. (2000).

2. Materials and methods

2.1. The M. officinalis preparation

A standardised, commercial extract of *M. officinalis* prepared by Pharmaton (Lugano, Switzerland) was utilised in the current study. Standardisation and conformity of the extract is assured by strict in-process controls during manufacture and complete analytical control of the resulting dry extract. The production method involves dried leaves of *M. officinalis* being reduced to fragments and extracted up to exhaustion in a 30:70 methanol/water mixture. The resultant liquid extract is evaporated and homogenised to yield a soft extract, to which inert processing agents (dried glucose syrup and colloidal anhydrous silicon dioxide to 7% and 3% of the final dried weight, respectively) are added. This mixture is homogenised and taken to dryness, ground, mixed and sieved.

2.2. Cholinergic receptor binding and chemical analysis

In order to provide a valid comparison with previous studies assessing the cholinergic receptor binding properties of *M. officinalis* leaf (Wake et al., 2000), the IC_{50} concentrations for the displacement of [^{3}H]-(*N*)-nicotine from nicotinic receptor and [^{3}H]-(*N*)-scopolamine from muscarinic receptors were established in human occipital cortex tissue using an identical extraction and receptor methodology to that previously used (for details, see Wake et al., 2000). The extract was also analysed using gas chromatograph mass spectroscopy (GCMS) for terpene constituents.

2.3. Cognitive assessment

2.3.1. Participants

Fifteen female and five male undergraduate volunteers (mean age 19.2 years, range 18–22 years) took part in the study, which was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority. Prior to participation, each volunteer signed an informed consent form and completed a medical health questionnaire. All participants reported that they were in good health and were taking no illicit social drugs. Additionally, they were free of any "over the counter" or prescribed medications, with the exception, for some female volunteers, of the contraceptive pill. Habitual smokers were excluded from the study. Of the 20 participants, 2 were occasional, light, social smokers (average consumption <2 cigarettes a day in both cases) and they agreed to abstain from smoking from rising on the day of the study until after completion of testing. All participants abstained from caffeine-containing products throughout each study day and alcohol for a minimum of 12 h prior to the first testing session of the morning. Participants were asked to eat their normal breakfast and a light lunch. Volunteers were paid £75 for participating in the study.

2.3.2. Cognitive measures

2.3.2.1. CDR computerised assessment battery. The CDR computerised assessment battery (Wesnes et al., 1987) has been used in hundreds of European and North American drug trials and has been shown to be sensitive to acute cognitive improvements (e.g. Moss et al., 1998; Scholey et al., 1999) as well as impairments with a wide variety of substances (e.g. Ebert et al., 1998; O'Neill et al., 1995).

A tailored version of the CDR battery was used. This has previously been found to be sensitive to improved cognitive function as a consequence of acute ingestion of both *G. biloba* (Kennedy et al., 2000) and *P. ginseng* (Kennedy et al., 2001a) and acute and chronic administration of a *G. biloba*/*P. ginseng* combination (Kennedy et al., 2001b; Wesnes et al., 1997, 2000). The selection of computer-controlled tasks from the system was administered with parallel forms of the tests being presented at each testing session. Presentation was via desktop computers with high-resolution VGA colour monitors. With the exception of written word recall tests, all responses were recorded via two-button (YES/NO) response boxes. The entire selection of tasks took approximately 20 min.

Tests were administered in the following order:

Word Presentation: Fifteen words, matched for frequency and concreteness, were presented in sequence on the monitor for the participant to remember. Stimulus duration was 1 s, as was the interstimulus interval.

Immediate Word Recall: The participant was allowed 60 s to write down as many of the words as possible. The task was scored as number of words produced minus errors and intrusions, and the resulting score was converted into a percentage.

Picture Presentation: A series of 20 photographic images of everyday objects and scenes were presented on the monitor at the rate of 1 every 3 s, with a stimulus duration of 1 s, for the participant to remember.

Simple Reaction Time: The participant was instructed to press the YES response button as quickly as possible every time the word YES was presented on the monitor. Fifty stimuli were presented with an interstimulus interval that varied randomly between 1 and 3.5 s. Reaction times were recorded in milliseconds.

Digit Vigilance Task: A target digit was randomly selected and constantly displayed to the right of the monitor screen. A series of digits was presented in the centre of the screen at the rate of 80 per minute and the participant was required to press the YES button as quickly as possible every time the digit in the series matched the target digit. The task lasted 1 min and there were 15 stimulus–target matches. Task measures were accuracy (%), reaction time (ms) and number of false alarms.

Choice Reaction Time: Either the word NO or the word YES was presented on the monitor and the participant was required to press the corresponding button as quickly as possible. There were 50 trials, of which the stimulus word was chosen randomly with equal probability, with a randomly varying interstimulus interval of between 1 and 3.5 s. Reaction times (ms) and accuracy (%) were recorded.

Spatial Working Memory: A pictorial representation of a house was presented on the screen with four of its nine windows lit. The participant was instructed to memorise the position of the illuminated windows. In 36 subsequent presentations of the house, one of the windows was illuminated and the participant decided whether or not this matched one of the lighted windows in the original presentation. The participant made their response by pressing the YES or NO response button as quickly as possible. Mean reaction times were measured in milliseconds and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages, which were used to derive a "percent greater than chance performance" score.

Numeric Working Memory: Five digits were presented sequentially for the participant to hold in memory. This

was followed by a series of 30 probe digits for each of which the participant decided whether or not it had been in the original series and pressed the YES or NO response button as appropriate as quickly as possible. This was repeated two further times with different stimuli and probe digits. Mean reaction times were measured in milliseconds and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages, which were used to derive a "percent greater than chance performance" score.

Delayed Word Recall: The participant was again given 60 s to write down as many of the words as possible. The task was scored as number correct, errors and intrusions and the resulting score was converted into a percentage.

Delayed Word Recognition: The original words plus 15 distractor words were presented one at a time in a randomised order. For each word, the participant indicated whether or not he recognised it as being included in the original list of words by pressing the YES or NO button as appropriate and as quickly as possible. Mean reaction times were measured in milliseconds and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages, which were used to derive a "percent greater than chance performance" score.

Delayed Picture Recognition: The original pictures plus 20 distractor pictures were presented one at a time in a randomised order. For each picture, participants indicated whether or not it was recognised as being from the original series by pressing the YES or NO button as appropriate and as quickly as possible. Mean reaction times were measured in milliseconds and accuracy of responses to both original and novel (distractor) stimuli were recorded as percentages, which were used to derive a "percent greater than chance performance" score.

Primary cognitive outcome measures. The above measures were collapsed into the four global outcome factors derived from the battery by factor analysis (see Wesnes et al., 2000 for details), as previously utilised by Kennedy et al. (2000) and Wesnes et al. (1997, 2000), with two further memory subfactors ("secondary" and "working" memory) as utilised by Kennedy et al. (2001a,b). The contribution of each individual task outcome to the outcome factors is represented in Fig. 1.

Accuracy of performance

Attention. Accuracy of Attention is derived by calculating the combined percentage accuracy across the choice reaction time and digit vigilance tasks with adjustment for false alarms from the latter test and 100% accuracy across the two tasks would generate a maximum score of 100.

Memory. Quality of Memory is derived by combining the percentage accuracy scores (adjusted for proportions of novel and original stimuli where appropriate) from all of the working and secondary memory tests—spatial working memory, numeric working memory, word recognition, picture recognition, immediate word recall and delayed word recall (with adjustments to the total percent correct for errors and intrusions on the latter two tasks) and 100% accuracy across the six tasks would generate a maximum score of 600 on this index.

Secondary Memory subfactor is derived by combining the percentage accuracy scores (adjusted for proportions of novel and original stimuli where appropriate) from all of the secondary memory tests—word recognition, picture recognition, immediate word recall and delayed word recall (with adjustments to the total percent correct for errors and intrusions on the latter two tasks) and 100% accuracy across the four tasks would generate a maximum score of 400 on this index.

Working Memory subfactor is derived by combining the percentage accuracy scores from the two working memory tests—spatial working memory and numeric working memory—and 100% accuracy across the two tasks would generate a maximum score of 200 on this index.

Speed of performance

Attention. Speed of Attention is derived by combining the reaction times of the three attentional tasks—simple reaction time, choice reaction time and digit vigilance (units are summed milliseconds for the three tasks).

Memory. Speed of Memory is derived by combining the reaction times of the four computerised memory tasks—numeric working memory, spatial memory, delayed word recognition and delayed picture recognition (units are summed milliseconds for the four tasks).

2.3.2.2. Serial subtraction tasks. *Serial sevens.* A modified computerised version of the Serial Sevens test was utilised. The original verbal Serial Sevens test (Hayman, 1942) has appeared in a number of forms, including as part of the Mini-Mental State Examination (Folstein et al., 1975). It has been used to assess cognitive impairment during hypoglycaemia (e.g. Hale et al., 1982; Taylor and Rachman, 1987) and has also been used to investigate the relationship between increased blood glucose levels and cognitive performance (Kennedy and Scholey, 2000; Scholey, 2001; Scholey et al., 2001).

In the current study, computerised versions of the serial subtractions tasks were implemented (see Scholey et al., 2001 for details) here using tests of 2-min duration. For the Serial Sevens task, a standard instruction screen informed the participant to count backwards in sevens from the given number, as quickly and accurately as possible, using the numeric keypad to enter each response. Participants were also instructed verbally that if they were to make a mistake they should carry on subtracting from the new incorrect number. A random starting number between 800 and 999 was presented on the computer screen, which was cleared by the entry of the first response. Each three-digit response was entered via the numeric keypad, with each digit being represented on screen by an asterisk. Pressing the enter key signalled the end of each response and cleared the three asterisks from the screen. The task was scored for total number of subtraction and number of errors. In

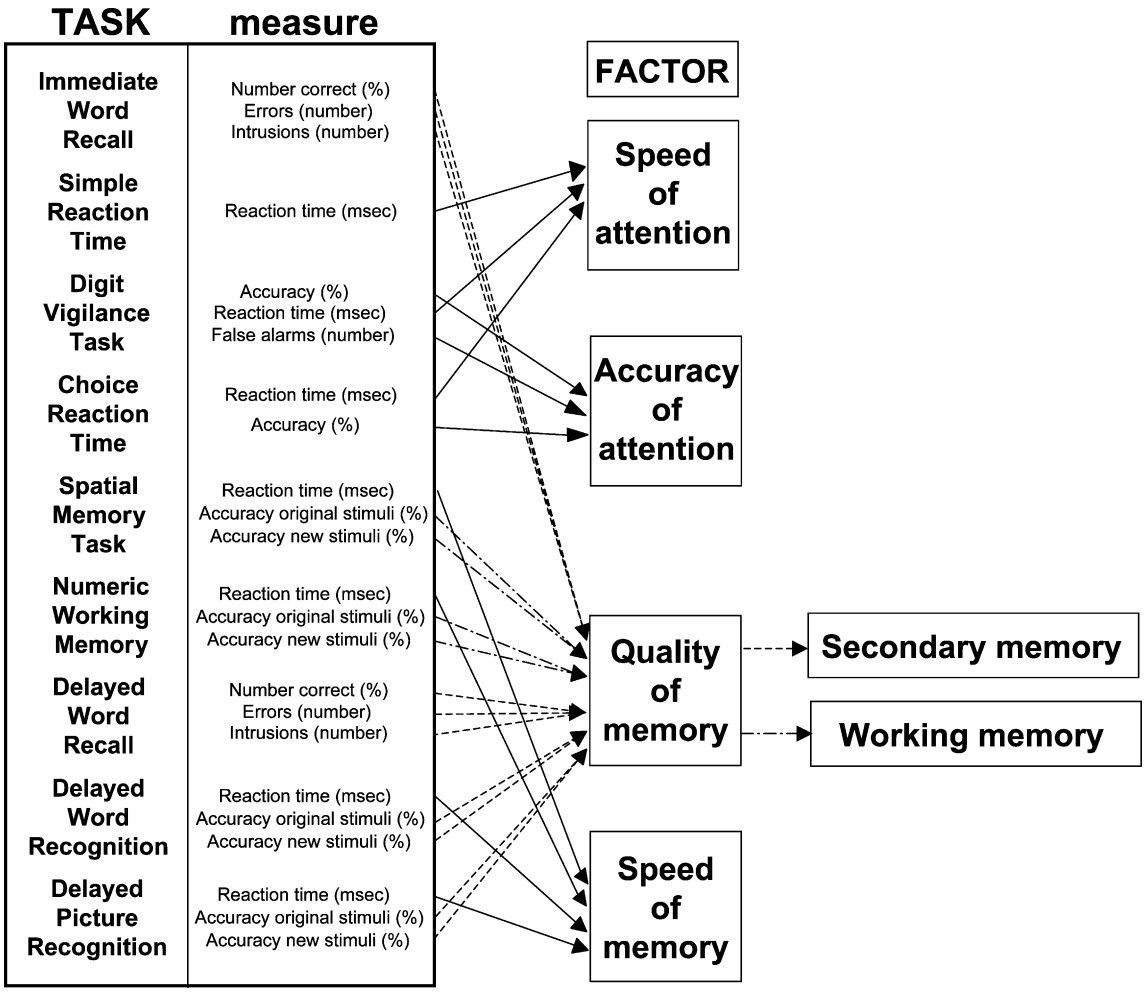

Fig. 1. Schematic representation of the CDR battery showing (from left to right) running order of tasks, individual task outcome measures and the composition of the four factors derived by factor analysis. Arrows indicate that a task outcome measure contributes to the given factor: Speed of Attention, Accuracy of Attention, Quality of Memory or Speed of Memory. Differential dotted lines indicate contribution to both Quality of Memory and to either Working Memory or Secondary Memory (adapted from Kennedy et al., 2000).

the case of incorrect responses, subsequent responses were scored as positive if they were correct in relation to the new number.

The Serial Threes task was identical to Serial Sevens, except that it involved serial subtraction of threes.

2.3.2.3. Subjective mood measure. The Bond–Lader Visual Analogue Scales (Bond and Lader, 1974). The 16 visual analogue scales of Bond–Lader were combined as recommended by the authors to form three mood factors: "alert," "calm" and "contented."

2.3.3. Treatments

On each study day, participants received six capsules of identical appearance, each containing either 150 mg of *M. officinalis* extract or a placebo (inert processing additives plus sucrose). The manufacturers suggest that a typical daily dose of this *M. officinalis* extract would be 600 mg. Therefore, depending on the condition to which they were allocated on that particular day, the combination of capsules corresponded to a dose of either 0 (placebo), 300, 600 or 900 mg of *M. officinalis* extract.

2.3.4. Procedure

Each participant was required to attend a total of 5 study days that were conducted 7 days apart to ensure a sufficient wash-out between conditions. Testing took place in a suite of laboratories, with participants visually isolated from each other.

On arrival at their first session on the first day, participants were randomly allocated to a treatment regime using a Latin square design, which counterbalanced the order of treatments across the 4 active days of the study.

The first day was identical to the following four, except that no treatment (active or placebo) was offered to allow familiarisation with the test battery and procedure. Data from the five sessions of this practice day were not included in any analysis.

Each study day comprised five identical testing sessions. The first was a pre-dose testing session, which established

Table 1
Effects of *M. officinalis* on individual task outcome measures from the CDR battery

Measure		Pre-dose baseline score	Post-dose change from baseline score			
			1 h	2.5 h	4 h	6 h
Immediate word recall (percent accuracy)	Placebo	49.00 ± 4.16	− 2.33 ± 3.32	− 3.17 ± 4.25	− 0.50 ± 4.61	− 2.67 ± 3.93
	300 mg	47.00 ± 3.32	− 0.83 ± 3.14	3.50 ± 3.49	− 1.33 ± 4.18	− 2.67 ± 3.72
	600 mg	50.17 ± 3.79	− 1.83 ± 2.92	− 2.67 ± 2.58	− 4.00 ± 3.89	− 3.33 ± 3.48
	900 mg	47.83 ± 3.54	− 3.50 ± 5.11	− 8.50 ± 3.46	− 5.33 ± 5.03	− 4.67 ± 3.54
Simple reaction time (ms)	Placebo	267.57 ± 7.24	4.99 ± 8.56	8.87 ± 6.91	5.15 ± 10.30	10.58 ± 9.13
	300 mg	266.19 ± 9.56	4.55 ± 4.86	2.75 ± 5.65	18.93 ± 10.10	31.92 ± 17.79
	600 mg	263.17 ± 6.98	11.95 ± 6.70	6.51 ± 7.87	15.76 ± 9.41	19.65 ± 5.68
	900 mg	262.86 ± 4.68	17.33 ± 9.16	14.96 ± 8.84	21.23 ± 11.05	29.07 ± 13.34
Digit vigilance accuracy (%)	Placebo	96.67 ± 1.03	− 1.33 ± 1.72	0.67 ± 1.27	− 2.00 ± 1.88	1.67 ± 1.27
	300 mg	97.67 ± 0.88	− 1.33 ± 1.33	− 2.00 ± 1.46	− 1.33 ± 1.42	− 0.33 ± 1.13
	600 mg	94.67 ± 1.42	3.33 ± 1.23****	2.00 ± 1.46	3.00 ± 1.23*****	3.00 ± 1.41
	900 mg	97.00 ± 1.13	− 0.67 ± 1.07	0.00 ± 1.45	− 0.33 ± 1.23	− 0.67 ± 1.60
Digit vigilance false alarms (number)	Placebo	0.45 ± 0.15	0.40 ± 0.24	0.15 ± 0.28	0.00 ± 0.19	0.10 ± 0.14
	300 mg	0.60 ± 0.20	− 0.05 ± 0.21	− 0.05 ± 0.21	0.00 ± 0.27	0.00 ± 0.30
	600 mg	0.60 ± 0.15	− 0.20 ± 0.20	0.15 ± 0.27	− 0.20 ± 0.21	− 0.30 ± 0.22
	900 mg	0.65 ± 0.13	− 0.30 ± 0.16	0.20 ± 0.25	− 0.15 ± 0.20	− 0.10 ± 0.20
Digit vigilance reaction time (ms)	Placebo	396.68 ± 7.91	− 1.87 ± 7.48	1.23 ± 6.89	14.36 ± 9.83	12.02 ± 8.16
	300 mg	397.20 ± 6.72	1.46 ± 8.21	− 0.32 ± 7.47	3.91 ± 7.27	12.20 ± 7.49
	600 mg	396.29 ± 6.99	0.78 ± 9.35	1.86 ± 8.16	7.47 ± 9.66	14.26 ± 10.42
	900 mg	398.63 ± 6.63	3.93 ± 7.63	5.56 ± 8.75	2.52 ± 7.32	20.40 ± 8.83
Choice reaction time accuracy (%)	Placebo	95.00 ± 1.00	− 1.60 ± 0.75	− 2.80 ± 0.96	− 2.20 ± 1.04	− 2.60 ± 1.13
	300 mg	94.70 ± 0.91	0.50 ± 0.82*	− 1.70 ± 1.08	0.30 ± 1.03**	− 2.90 ± 1.01
	600 mg	94.10 ± 1.24	0.20 ± 1.12	− 0.70 ± 1.34*	− 1.00 ± 0.98	− 1.00 ± 1.14
	900 mg	94.10 ± 0.90	− 0.80 ± 1.22	− 0.60 ± 0.88*	− 1.50 ± 1.17	0.40 ± 1.00***
Choice reaction time (ms)	Placebo	425.04 ± 12.62	4.56 ± 13.15	− 5.81 ± 7.69	− 3.21 ± 7.50	− 2.59 ± 9.80
	300 mg	437.94 ± 19.91	− 9.39 ± 8.37	− 17.87 ± 8.38	− 13.22 ± 10.15	− 10.27 ± 17.96
	600 mg	418.45 ± 8.82	3.23 ± 5.78	− 2.61 ± 8.65	8.27 ± 9.40	9.74 ± 7.37
	900 mg	431.63 ± 12.12	1.57 ± 9.09	2.58 ± 11.03	− 4.00 ± 11.10	− 4.93 ± 9.20
Spatial memory (percent greater than chance)	Placebo	85.31 ± 5.05	6.50 ± 5.20	2.31 ± 5.97	1.75 ± 5.50	3.56 ± 6.50
	300 mg	91.56 ± 2.74	− 1.06 ± 2.54	− 10.31 ± 6.38***	0.25 ± 2.47	− 5.56 ± 5.48*
	600 mg	93.94 ± 1.21	− 1.25 ± 1.74	− 10.75 ± 3.61***	− 0.44 ± 1.85	− 4.19 ± 1.65
	900 mg	92.25 ± 1.60	− 4.25 ± 3.91*	− 6.69 ± 4.59*	− 2.50 ± 2.17	− 6.38 ± 3.69*
Spatial memory reaction time (ms)	Placebo	603.16 ± 28.60	− 17.33 ± 27.98	− 52.78 ± 23.46	− 48.77 ± 21.52	− 61.10 ± 24.94
	300 mg	595.81 ± 30.12	− 16.51 ± 23.23	− 39.30 ± 23.39	− 44.72 ± 28.12	− 60.22 ± 20.27
	600 mg	592.01 ± 28.91	− 16.71 ± 15.85	− 28.33 ± 19.69	− 36.91 ± 21.97	− 7.95 ± 25.58
	900 mg	599.03 ± 29.68	− 35.61 ± 22.24	− 27.07 ± 20.13	− 20.11 ± 20.46	− 45.01 ± 20.82
Numeric working memory (percent greater than chance)	Placebo	84.33 ± 2.66	− 2.11 ± 2.75	− 1.00 ± 2.81	− 4.11 ± 2.51	− 6.55 ± 2.81
	300 mg	87.00 ± 2.58	− 6.89 ± 2.23	− 1.22 ± 1.70	− 3.56 ± 1.75	− 4.22 ± 1.64
	600 mg	86.00 ± 2.38	− 1.44 ± 3.14	− 3.00 ± 2.59	− 3.44 ± 2.36	− 2.89 ± 1.66
	900 mg	86.00 ± 2.64	− 5.67 ± 1.83	− 4.89 ± 1.85	− 5.89 ± 2.79	− 7.11 ± 2.50
Numeric working memory reaction time (ms)	Placebo	515.88 ± 20.52	5.17 ± 9.23	− 14.86 ± 8.48	− 8.85 ± 6.22	− 23.80 ± 12.41
	300 mg	523.64 ± 17.51	− 6.09 ± 11.95	− 0.01 ± 10.82	− 9.01 ± 8.79	− 22.12 ± 13.05
	600 mg	548.97 ± 22.47	− 11.09 ± 10.32	− 11.98 ± 11.70	− 37.07 ± 9.00	− 20.15 ± 9.70
	900 mg	522.74 ± 18.28	6.56 ± 9.17	4.28 ± 12.31	− 3.99 ± 14.21	− 31.76 ± 14.61
Delayed word recall (percent accuracy)	Placebo	36.67 ± 3.03	− 15.33 ± 2.95	− 13.50 ± 3.28	− 13.67 ± 2.56	− 17.67 ± 3.33
	300 mg	37.50 ± 3.00	− 9.50 ± 2.42	− 12.33 ± 4.24	− 14.50 ± 4.27	− 15.83 ± 3.26
	600 mg	36.17 ± 3.24	− 10.33 ± 3.44	− 8.33 ± 2.30	− 11.00 ± 3.14	− 17.00 ± 2.81
	900 mg	36.67 ± 3.57	− 11.00 ± 3.60	− 17.83 ± 3.02	− 23.00 ± 4.72	− 16.83 ± 3.80

(continued on next page)

Table 1 (*continued*)

Measure		Pre-dose baseline score	Post-dose change from baseline score			
			1 h	2.5 h	4 h	6 h
Word recognition (percent greater than chance)	Placebo	50.33 ± 5.81	5.00 ± 3.90	6.33 ± 4.57	5.67 ± 6.95	2.33 ± 6.16
	300 mg	59.33 ± 4.61	− 2.67 ± 3.76	− 4.33 ± 5.24	− 10.67 ± 4.85***	− 8.33 ± 5.34
	600 mg	65.71 ± 5.18	− 9.37 ± 4.66**	− 14.04 ± 4.74*****	− 16.71 ± 6.00*****	− 20.04 ± 5.42*****
	900 mg	53.67 ± 5.87	4.33 ± 5.67	− 9.58 ± 6.39***	− 2.67 ± 4.06	− 6.27 ± 4.60
Word recognition reaction time (ms)	Placebo	680.21 ± 37.77	− 18.69 ± 35.35	− 15.97 ± 38.67	− 3.79 ± 41.75	− 37.96 ± 37.87
	300 mg	667.84 ± 21.92	23.70 ± 22.81	12.58 ± 22.52	− 2.88 ± 19.46	− 0.80 ± 17.08
	600 mg	663.26 ± 22.29	10.25 ± 17.85	− 0.49 ± 21.12	− 4.05 ± 19.63	9.70 ± 23.88
	900 mg	664.99 ± 22.67	1.52 ± 20.25	24.73 ± 18.00	6.20 ± 21.79	− 6.16 ± 22.28
Picture recognition (percent greater than chance)	Placebo	66.50 ± 5.67	− 0.75 ± 6.01	− 1.50 ± 7.45	− 0.50 ± 6.58	− 12.50 ± 8.19
	300 mg	68.50 ± 5.59	− 6.50 ± 4.91	− 10.25 ± 4.35	− 5.25 ± 3.49	− 13.00 ± 3.67
	600 mg	69.00 ± 4.27	− 4.25 ± 4.39	− 5.25 ± 4.52	− 14.50 ± 4.76	− 0.25 ± 3.65
	900 mg	67.75 ± 4.36	− 8.00 ± 3.25	− 8.00 ± 3.02	− 11.50 ± 3.48	− 13.75 ± 4.17
Picture recognition reaction time (ms)	Placebo	741.88 ± 25.36	− 7.84 ± 14.85	− 10.32 ± 18.62	2.92 ± 17.00	− 14.37 ± 19.19
	300 mg	738.49 ± 25.81	− 0.45 ± 21.00	11.19 ± 16.95	− 7.11 ± 15.22	− 20.44 ± 25.27
	600 mg	748.99 ± 23.03	12.56 ± 17.00	0.35 ± 20.83	2.33 ± 20.42	9.53 ± 16.11
	900 mg	741.83 ± 26.46	13.77 ± 14.37	9.34 ± 21.55	17.31 ± 23.83	0.40 ± 17.37

Mean baseline and change from baseline scores are presented (with standard errors). Asterisks denote results of planned comparisons on the measures (shown in italics) that showed a main effect of treatment.

* P=.05 compared to placebo.

** P=.01 compared to placebo.

*** P=.005 compared to placebo.

**** P=.001 compared to placebo.

***** P=.0005 compared to placebo.

baseline performance for that day and was immediately followed by consumption of the day's treatment on visits 2–5. Further testing sessions began at 1, 2.5, 4 and 6 h following consumption of the day's treatment.

Each testing session comprised completion of the Bond–Lader Visual Analogue Scales, the CDR test battery and finally the Serial Threes and Serial Sevens computerised subtraction tasks.

2.3.5. Statistics

Scores on the individual task outcomes, the four primary factors and the two memory subfactors were analysed as ''change from baseline'' using the SAS statistical package.

Prior to carrying out planned comparisons, an analysis of variance (ANOVA) (PROC GLM), with terms fitted to the model for Dose, Visit, Dose × Visit and Subject (Kirk, 1968), was carried out to identify main effects and interaction effects on each measure. The primary statistical analysis followed the recommendation of Kepple (1991) and was carried out using planned comparisons, which were made between placebo and each of the three doses of *M. officinalis* (300, 600 and 900 mg) at each time point, utilising t tests with the mean squares for Dose × Time × Subjects from an omnibus ANOVA (PROC GLM) as an error term. To ensure the overall protection level, only those planned comparisons associated with measures that generated a significant main effect or interaction effect are reported. Furthermore, all testings were two tailed. Comparisons were strictly planned prior to the study and were restricted to the number of conditions minus one at each time point. Only probabilities associated with these pre-planned comparisons were calculated.

3. Results

3.1. Cholinergic receptor binding analysis

The IC_{50} concentrations for nicotinic and muscarinic receptor binding to human occipital cortex tissue of extracts of the encapsulated material were 11 and 4 mg/ml, respectively.

It was not possible to extract sufficient material from the capsule contents for GCMS analysis of terpene content.

3.2. Cognitive measures

Prior to analysis of change from baseline data, mean pre-dose raw baseline scores for all four conditions (placebo, 300, 600 and 900 mg *M. officinalis*) for each outcome (individual task scores, cognitive factor scores, serial subtraction scores and mood scale scores) were subjected to a one-way, repeated-measures ANOVA. There were no significant differences on any measure.

3.2.1. Individual task outcome measures

Mean pre-dose baseline raw scores and change from baseline scores for each condition at each post-dose time

Measure		Pre-dose Baseline score	Post-dose change from baseline score 1 hour	2.5 hours	4 hours	6 hours
Speed of Attention (summed msecs)	placebo	372.15 17.20	7.69 24.95	4.30 15.77	16.30 19.68	20.01 18.75
	300mg	390.90 15.55	-3.38 15.44	-15.43 15.09	9.62 21.47	33.84 34.56
	600mg	400.98 13.49	15.96 13.83	5.76 15.69	31.50 23.11	43.65 15.79
	900mg	384.17 14.75	36.80 26.13	36.14 26.36	33.76 31.19	58.55 35.76
Accuracy of Attention (%)	***placebo***	***90.55 0.91***	***-1.80 0.95***	***-1.25 0.63***	***-2.00 1.05***	***-0.65 0.96***
	300mg	***90.70 0.65***	***-0.30 0.77***	***-1.70 0.95***	***-0.45 0.89***	***-1.60 0.77***
	600mg	***89.05 0.86***	***1.80 0.78**********	***0.40 1.04****	***1.05 0.84**********	***1.15 0.98****
	900mg	***90.05 0.66***	***-0.40 0.71***	***-0.50 0.76***	***-0.75 0.80***	***0.00 0.85***
Speed of Memory (summed msecs)	placebo	2541. 92.35	-38.69 41.89	-93.93 63.18	-58.49 53.35	-137.2 68.23
	300mg	2525.7 81.32	0.65 60.50	-15.55 52.35	-63.71 46.12	-103.6 64.18
	600mg	2553.2 85.00	-4.99 40.32	-40.44 55.43	-75.70 48.99	-8.87 52.50
	900mg	2528.6 82.94	-13.77 34.20	11.28 40.45*	-0.59 50.29	-82.53 44.78
Quality of Memory (% X 6)	***placebo***	***372.15 17.20***	***-9.03 13.17***	***-10.52 14.39***	***-11.36 19.68***	***-33.49 20.95***
	300mg	***390.90 15.55***	***-27.45 10.76***	***-34.95 14.03***	***-35.06 12.00***	***-49.62 10.28***
	600mg	***400.98 13.49***	***-28.48 8.85***	***-44.04 9.29*****	***-50.09 12.30******	***-47.70 9.63***
	900mg	***384.17 14.75***	***-28.08 14.99***	***-55.49 13.41********	***-50.89 13.05******	***-55.01 11.78***
Secondary Memory (% X 4)	***placebo***	***202.50 13.71***	***-13.42 11.14***	***-11.83 12.62***	***-9.00 15.48***	***-30.50 16.54***
	300mg	***212.33 12.66***	***-19.50 9.10***	***-23.42 13.53***	***-31.75 12.31****	***-39.83 8.46***
	600mg	***221.04 11.99***	***-25.79 8.43***	***-30.29 7.69***	***-46.21 11.31******	***-40.62 8.59***
	900mg	***205.92 13.14***	***-18.17 12.51***	***-43.92 11.50******	***-42.50 11.70******	***-41.52 9.12***
Working Memory (% X 2)	***placebo***	***169.65 6.63***	***4.39 6.44***	***1.31 7.42***	***-2.36 6.86***	***-2.99 7.92***
	300mg	***178.56 4.74***	***-7.95 3.72 ****	***-11.53 6.83 ****	***-3.31 2.71***	***-9.78 5.22***
	600mg	***179.94 2.97***	***-2.69 3.75***	***-13.75 4.78 ******	***-3.88 3.05***	***-7.07 2.40***
	900mg	***178.25 3.62***	***-9.92 4.97 ******	***-11.58 4.92 ****	***-8.39 3.38***	***-13.49 5.10 ****

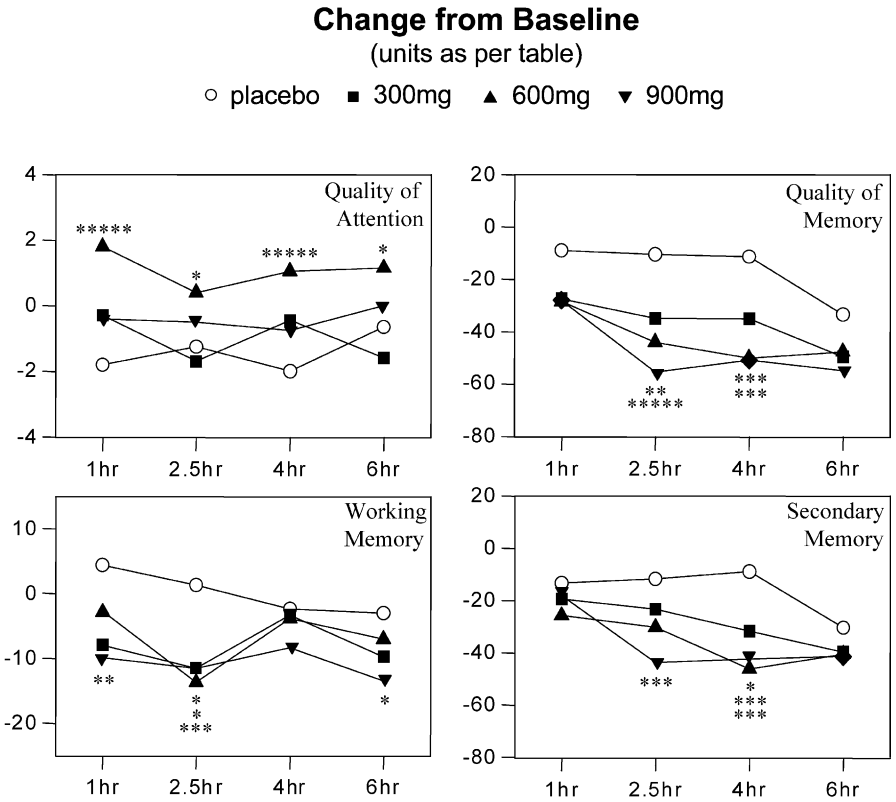

Fig. 2. Effects of *M. officinalis* on cognitive factors: Speed of Attention, Accuracy of Attention, Speed of Memory, Quality of Memory, Secondary Memory and Working Memory. The table presents means (with standard errors) of baseline scores and change from baseline scores for each dose of *M. officinalis*. Asterisks denote results of planned comparisons on the measures (shown in bold italics) that showed a main effect of treatment. Graphs represent the change from baseline scores for the relevant outcome measure (**P*=.05, ** *P*=.01, *** *P*=.005, *****P*=.001, ***** *P*=.0005 compared to the corresponding placebo score).

point on the individual task outcome measures are represented in Table 1.

3.2.2. Primary outcome measures

Mean raw baseline scores and change from baseline factor scores for each condition across each session are represented in the tables and graphs of Fig. 2.

3.2.2.1. Attention. Accuracy of Attention factor: There was a significant main effect of treatment on performance of the tasks making up the Accuracy of Attention factor [$F(3,285)=7.14$, $P=.0001$]. Planned comparisons revealed that performance was significantly improved for the 600-mg dose of *M. officinalis* at all time points: 1 h [$t(171)=4.32$, $P=.0001$], 2.5 h [$t(171)=1.98$, $P=.049$], 4 h [$t(171)=3.66$, $P=.0003$] and 6 h [$t(171)=2.16$, $P=.03$]. Inspection of the component measures revealed that accuracy scores on both the Choice reaction time task [$F(3,285)=2.58$, $P=.05$] and the Digit vigilance task [$F(3,285)=7.26$, $P=.0001$] evinced a significant main effect of treatment. Planned comparisons showed that whilst significant improvements on Digit vigilance accuracy were restricted to the 600-mg dose, with improvements at 1 h [$t(171)=3.35$, $P=.001$] and 4 h post-dose [$t(171)=3.59$, $P=.0004$], all doses evinced improvements on Choice reaction time accuracy, with significant improvements for 300 mg at 1 h [$t(171)=2.2$, $P=.029$] and 4 h post-dose [$t(171)=2.62$, $P=.01$], for 600 mg at 2.5 h post-dose [$t(171)=2.2$, $P=.029$] and for 900 mg at 2.5 h [$t(171)=2.3$, $P=.022$] and 4 h post-dose [$t(171)=3.14$, $P=.002$].

Speed of Attention factor: There were no significant main or interaction effects for either the Speed of Attention factor or its component tasks.

3.2.2.2. Memory. Quality of Memory factor: There was a main effect of treatment on the performance of the Quality of Memory factor [$F(3,285)=4.67$, $P=.003$]. Planned comparisons revealed significant decrements in the accuracy of memory task performance, in comparison to placebo, for both 600 and 900 mg of *M. officinalis* at 2.5 h [$t(171)=2.63$, $P=.009$ and $t(171)=3.53$, $P=.0005$, respectively] and at 4 h post-dose [$t(171)=3.03$, $P=.0028$ and $t(171)=3.01$, $P=.0023$, respectively].

Secondary Memory factor: Performance on the Secondary Memory factor evinced a significant main effect of treatment [$F(3,285)=2.9$, $P=.04$]. Planned comparisons showed that whilst the highest dose alone showed a decrement on this factor at the 2.5 h testing session [$t(171)=2.83$, $P=.005$], all three doses of *M. officinalis* resulted in significant impairment at the 4 h testing session [300 mg $t(171)=2.01$, $P=.046$, 600 mg $t(171)=3.29$, $P=.0012$ and 900 mg $t(171)=2.96$, $P=.0035$].

Comparison of the individual task outcome scores suggests that the overall effects of treatment only reached significance for the Word recognition task [$F(3,285)=10.33$, $P<.0001$]. On this task, performance was significantly disturbed at all time points for the 600-mg dose [1 h $t(171)=2.61$, $P=.009$, 2.5 h $t(171)=3.7$, $P=.0003$, 4 h $t(171)=4.08$, $P=.00007$ and 6 h $t(171)=4.07$, $P=.00007$]. The 300-mg dose evinced a similar pattern with decrements

Mood Factor		Pre-dose Baseline score	Post-dose change from baseline score			
			1 hour	2.5 hours	4 hours	6 hours
ALERT	placebo	***50.37*** $_{3.85}$	***9.01*** $_{3.52}$	***6.85*** $_{3.98}$	***10.58*** $_{4.36}$	***11.07*** $_{4.30}$
	300mg	***53.40*** $_{4.26}$	***6.63*** $_{3.47}$	***10.39*** $_{4.60}$	***7.06*** $_{3.02}$	***5.65*** $_{3.59}$ *
	600mg	***50.89*** $_{4.70}$	***5.14*** $_{2.94}$	***4.44*** $_{3.48}$	***6.15*** $_{3.83}$	***6.85*** $_{3.46}$
	900mg	***53.09*** $_{3.84}$	***-0.83*** $_{2.89}$ ****	***0.92*** $_{3.97}$ *	***4.07*** $_{3.90}$ *	***1.45*** $_{4.34}$ ****
CONTENT	*placebo*	63.87 $_{3.72}$	-0.34 $_{3.27}$	2.28 $_{3.01}$	1.57 $_{3.92}$	2.88 $_{3.63}$
	300mg	68.29 $_{3.24}$	1.95 $_{1.93}$	1.18 $_{1.81}$	-2.19 $_{2.15}$	-1.24 $_{2.97}$
	600mg	62.87 $_{3.85}$	0.28 $_{3.03}$	-0.93 $_{2.84}$	1.97 $_{2.81}$	3.10 $_{3.74}$
	900mg	61.22 $_{2.71}$	2.33 $_{3.60}$	-1.13 $_{3.12}$	2.70 $_{2.72}$	-0.72 $_{3.13}$
CALM	***placebo***	***70.80*** $_{2.91}$	***-12.53*** $_{2.24}$	***-8.30*** $_{3.07}$	***-10.98*** $_{3.13}$	***-13.13*** $_{3.64}$
	300mg	***65.05*** $_{3.80}$	***-2.25*** $_{2.42}$ **	***-1.05*** $_{2.30}$ *	***-5.93*** $_{2.62}$	***-9.30*** $_{2.94}$
	600mg	***69.59*** $_{3.42}$	***-7.04*** $_{3.01}$	***-14.46*** $_{3.85}$	***-11.29*** $_{3.83}$	***-16.01*** $_{4.57}$
	900mg	***64.33*** $_{2.96}$	***-4.80*** $_{2.59}$ *	***-11.80*** $_{3.95}$	***-8.73*** $_{2.99}$	***-10.40*** $_{2.52}$

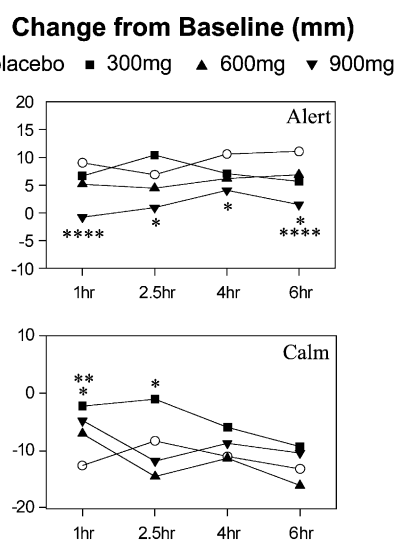

Fig. 3. Effects of *M. officinalis* on self-rated mood as measured using Bond–Lader Visual Analogue Scales. The table presents raw scores and change from baseline scores for each dose of *M. officinalis* (means with standard errors). Asterisks denote results of planned comparisons on the measures (shown in bold italics) that showed a main effect of treatment. Graphs represent the change from baseline scores for the three mood dimensions "alert," "calm" and "content" (*$P=.05$, ** $P=.01$, *** $P=.005$, ****$P=.001$, ***** $P=.0005$ compared to the corresponding placebo score).

that reached significance at 4 h [$t(171)=2.96$, $P=.0035$], with strong trends towards significant decrements at 2.5 h [$t(171)=1.94$, $P=.054$] and 6 h [$t(171)=1.94$, $P=.054$]. There was a single decrement associated with the 900-mg dose at the 2.5 h testing session [$t(171)=2.89$, $P=.004$].

Working Memory factor: There was also a significant main effect of treatment on the Working Memory factor [$F(3,285)=3.6$, $P=.01$]. Planned comparisons revealed that all three doses of *M. officinalis* resulted in significant decrements. At the 1 h post-dose testing session, both 300 mg [$t(171)=2.38$, $P=.018$] and 900 mg [$t(171)=2.76$, $P=.006$] evinced significant reductions in change from baseline scores. This was also true for all three doses at 2.5 h [300 mg $t(171)=2.47$, $P=.014$, 600 mg $t(171)=2.9$, $P=.0042$ and 900 mg $t(171)=2.49$, $P=.014$] and for the 900-mg dose at 6 h post-dose [$t(171)=2.02$, $P=.044$].

Analysis of the individual task scores suggested that this effect was isolated to the Spatial memory task [$F(3,285)=4$, $P<.008$], on which measure planned comparisons revealed that performance was significantly impaired for 300 mg at 2.5 and 6 h [$t(171)=2.89$, $P=.004$ and $t(171)=2.09$, $P=.038$, respectively], for 600 mg at 2.5 h post-dose [$t(171)=2.99$, $P=.003$] and for 900 mg at 1 h [$t(171)=2.47$, $P=.014$], 2.5 h [$t(171)=2.06$, $P=.041$] and 6 h post-dose [$t(171)=2.28$, $P=.024$].

Speed of Memory factor: There were no significant effects on this factor.

3.2.3. Serial subtraction tasks

There were no significant main effects or interactions on either of the serial subtraction tasks.

3.2.4. Subjective mood measures

Alert: There was a significant main effect of treatment on the "alert" factor derived from the Bond–Lader Visual Analogue Mood Scales [$F(3,285)=5.22$, $P<.002$]. Planned comparisons revealed that the 900-mg dose of *M. officinalis* was associated with a significant reduction in scores at all testing sessions [1 h $t(171)=3.81$, $P<.0002$, 2.5 h $t(171)=2.3$, $P=.023$, 4 h $t(171)=2.53$, $P<.012$ and 6 h $t(171)=3.73$, $P=.0003$, respectively]. The 300-mg dose resulted in a single significant reduction at the 6 h testing session [$t(171)=2.1$, $P=.037$].

Content: There was no modulation of the "content" factor.

Calm: ANOVA revealed a significant main effect of treatment on the "calm" factor derived from the mood scales [$F(3,285)=5.15$, $P<.002$]. Planned comparisons showed that, in comparison to placebo, ratings on the "calm" scale were significantly higher for both 300 and 900 mg at the 1 h testing session [$t(171)=3.13$, $P=.002$ and $t(171)=2.36$, $P=.019$, respectively]. The 300-mg dose was also associated with an increase on this scale at 2.5 h post-dose [$t(171)=2.21$, $P=.028$].

The effects of *M. officinalis* on the mood measures are presented in the table and graphs of Fig. 3.

4. Discussion

The results of the current study suggest that the ingestion of single doses of *M. officinalis* can modulate both the mood and the cognitive performance of healthy young volunteers in a dose- and time-dependent manner.

Improvement on the cognitive measures was restricted to the Accuracy of Attention factor, with benefits seen across all time points for the middle dose (600 mg) of *M. officinalis*. However, memory performance was disrupted for all doses of the extract, with relatively clear dose-related impairments on the global Quality of Memory measure and the Secondary Memory factor at the 2.5 and 4 h post-dose testing sessions. Decrements for all doses were also seen on the Working Memory factor, with these being most notable at the earlier testing sessions (1 and 2.5 h) and for the highest dose of *Melissa* (900 mg), which evinced reduced performance at all but the penultimate testing sessions.

Mood was also modulated, with significantly increased "calmness," in comparison to placebo, seen for the highest dose (900 mg) at the first testing session (1 h) and for the lowest dose (300 mg) at both of the first two testing sessions (1 and 2.5 h). Self-rated "alertness" was also reduced in comparison to placebo across all testing sessions for the highest dose (900 mg). The middle (600 mg) dose was not associated with any significant effects on mood.

The pattern of results can be viewed as largely consistent with both the contemporary use of *Melissa* as a calming agent and mild sedative (Kommission E Monograph, 1984; Bisset and Wichtl, 1994) and demonstrations of similar effects in both rodents (Wagner and Sprinkmeyer, 1973; Soulimani et al., 1991) and sufferers from severe dementia (Ballard et al., in press). Interestingly, the dose associated with the most positive modulation of mood (300 mg), with significantly increased scores on the Bond–Lader "calm" factor at the two earliest time points, was largely unaffected by the memory decrements associated with the other two doses. This may well suggest, in keeping with the herbalist's maxim that "less is more," that possible therapeutic doses lie below or at the lower end of the doses utilised here. Indeed, several smaller doses of *Melissa* throughout the day may be efficacious in its suggested role in the amelioration of dementia-related agitation (Perry et al., 1999).

In line with the notion that the lower dose was, on balance, the most beneficial, the middle dose was associated both with cognitive improvements on the Accuracy of Attention factor and decrements on the memory factors and with no modulation of mood. The highest dose, on the other hand, was detrimental throughout, with the most striking disturbance of memory processes coupled with reduced alertness throughout and possibly beyond the 6 h that testing encompassed.

Whilst the results here suggest that low doses may be of some utility in the beneficial modulation of mood and higher doses may well exert a mild sedative effect, there

is no evidence to support the historical role for *M. officinalis* in the enhancement of memory or the cholinergic properties of the plant (Perry et al., 1996; Wake et al., 2000). The cognitive effects seen here, albeit for different doses, include positive effects on attention and negative effects on memory, domains that would be expected to be modulated in the same direction in the case of cholinergic action (Feldman et al., 1997). It seems unlikely therefore that modulation of this neurotransmitter system underlies the effects seen here, and it is likely, as with all plant extracts, that any effects are as a consequence of several disparate mechanisms. In support of this, reference to the cholinergic binding properties evinced by this extract suggest that nicotinic receptor binding, with an IC_{50} concentration of 11 mg/ml, is much lower than in batches of fresh leaf assessed previously, for which IC_{50} values of between 0.08 and 3 mg/ml were obtained (Wake et al., 2000). Similarly, muscarinic receptor binding, with an IC_{50} concentration of 4 mg/ml, is towards the lower end of the range from the previous study, which reported IC_{50} values ranging from 0.5 to 5 mg/ml (Wake et al., 2000). It is possible that these low cholinergic binding properties are the result of a loss of volatile components during the manufacturing process, a possibility that is supported here by the inability to detect volatiles using GCMS. Alternatively, they may simply reflect a wide range of receptor binding properties in different batches of the plant.

Whilst the current study does not support a possible role for this specific extract of *M. officinalis* in the amelioration of the cholinergic disturbances associated with Alzheimer's disease (Perry et al., 1999), it does not preclude the possibility that an extract, oil or leaf of *M. officinalis* with the previously demonstrated human cortex cholinergic binding properties (Perry et al., 1996; Wake et al., 2000) may well be efficacious. Indeed, a treatment combining both calming effects and beneficial cholinergic modulation may well prove a novel treatment for Alzheimer's disease, especially given the lack of any known detrimental side effects associated with *M. officinalis*.

Given this first demonstration in humans of modulation of cognitive performance and mood as a consequence of ingestion of *M. officinalis*, the possibility that a cholinergically active *Melissa* will exert a more favourable profile of cognitive modulation in healthy young volunteers deserves investigation.

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