Improved working memory performance following administration of a single dose of American ginseng (*Panax quinquefolius* L.) to healthy middle-age adults

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Objective   A ginsenoside-rich extract of American ginseng (*Panax quinquefolius* L.), Cereboost™, was previously shown to improve working memory and mood in healthy young individuals. The present study represented a partial replication investigating whether these effects extended to healthy middle-aged individuals.
Methods   Fifty-two healthy volunteers (40–60 years old, mean age 51.63) received 200 mg of *P. quinquefolius* or a matching placebo according to a double-blind, placebo-controlled, balanced, crossover design. The Cognitive Drug Research battery and the Computerised Mental Performance Assessment System were used to evaluate cognitive performance at baseline then 1, 3 and 6 h following treatment. Blood glucose and mood were co-monitored.
Results   Compared with placebo, *P. quinquefolius* improved cognitive performance on 'Working Memory' factor at 3 h. Similar effects were observed in one of the two tasks making up this factor, spatial working memory. There were no significant effects on mood or blood glucose levels.
Conclusions   These data confirm that *P. quinquefolius* can acutely benefit working memory and extend the age range of this effect to middle-aged individuals. These changes are unlikely to be underpinned by modulation of blood glucose in this population. Copyright © 2015 John Wiley & Sons, Ltd.

INTRODUCTION

Extracts of ginseng have been recognised for decades for their effects on the central nervous system. They have both stimulatory and inhibitory effects (Kennedy and Scholey, 2003; Smith *et al.*, 2014) that may modulate neurotransmission, enhance memory and learning and provide neuroprotection. In a series of human trials, we have previously confirmed acute cognitive effects of Asian (*Panax*) ginseng (e.g. Kennedy *et al.*, 2001a; 2001b; Scholey and Kennedy, 2002; Reay *et al.*, 2005; 2006) and, more recently American ginseng *Panax quinquefolius* (Scholey *et al.*, 2010).

The main active constituents in ginseng extracts are believed to be the triterpenoid saponins, known as ginsenosides (Attele *et al.*, 1999). These can be classified into three groups on the basis of their chemical structure; the Panaxadiol group (Rb1, Rb2, Rb3, Rc, etc.), the Panaxatriol group (Re, Rf, Rg1, Rg2, Rh1) and the Oleanolic acid group (e.g. Ro). *P. quinquefolius* typically contains 4–6% (w/w) total ginsenosides, with ginsenosides Rb1, Re and Rd being the most abundant (Qi *et al.*, 2011). As with other herbal extracts, one challenge in this area relates to standardisation of treatment. It has been previously shown that there can be batch-to-batch variability in ginseng, with some extracts containing little or no ginsenosides (Harkey *et al.*, 2001; Sievenpiper *et al.*, 2004). When it comes to investigations of herbal substances in the psychopharmacology domain, it has been suggested that absence of the standardised herbal products on the market might have delayed the progress and consistency of the research in this area considerably and implementation of the standardised products provided a platform for systematic scientific investigations (Scholey *et al.*, 2005). Indeed, consistency of cognitive results

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obtained with *Panax ginseng* to some extent is due to the availability of its highly standardised extract (G115); hence, the availability of a standardised *P. quinquefolius* extract (Cereboost™) also provides an opportunity to commence a systematic assessment of its neurocognitive effects.

Cereboost™ is a dry native extract of *P. quinquefolius* L. roots with a high ginsenoside content, standardised to 10–12% total ginsenosides (Scholey *et al.*, 2010). This is up to threefold greater total ginsenosides than the *P. ginseng* extract G115. We recently reported findings of the randomised, double-blind, placebo-controlled, balanced, crossover trial evaluating the acute mood, neurocognitive and blood glucose effects of 100, 200 and 400 mg of Cereboost™ in young adults (Scholey *et al.*, 2010). The extract was associated with a significant and consistent improvement to working memory performance. Spatial working memory, assessed using Corsi blocks, was improved by all doses at all testing times (1, 3 and 6 h post treatment). There were also significant improvements to numeric working memory speed and immediate word recall associated with 200 mg dose. Alphabetic working memory speed was enhanced by 100 and 400 mg doses. There were also a number of time-specific and task-specific benefits associated with other doses. Self-rated calmness was increased following 100 mg dose at 3 and 6 h post treatment. Although potent glycaemic properties of *P. quinquefolius* has been previously reported (Vuksan *et al.*, 2000a, 2000b, 2000c), no treatment-related changes in glucose levels were observed in the previous study. Given that the glycaemic effects of *P. quinquefolius* (Vuksan *et al.*, 2000c) and the cognitive impact of blood glucose changes (for example a slightly older, middle-aged cohort, Messier *et al.*, 2010), may be more pronounced in individuals with poorer glucoregulation, blood glucose was co-monitored in the 40 to 60-year-old volunteers in the current study.

Normal ageing is associated with neurocognitive decline, which is likely underpinned by numerous systemic and central structural and functional changes. These include changes to neuronal connectivity, cellular calcium regulation, protein formation and destruction (proteolysis), neurotransmitter and hormonal changes and cerebral blood flow (Bishop *et al.*, 2010). Because these numerous biological changes lead to impairments during the course of ageing, it is likely that greater scope for cognitive enhancement exists in older populations. For example, the effects of other herbal extracts such as sage are greater in older than younger individuals (Scholey *et al.*, 2008), as are the effects of glucose administration (e.g. Macpherson

et al., 2014). Therefore, a logical step in evaluating the cognition-enhancing potential of pharmaceuticals, or nutraceuticals such as *P. quinquefolius* is to evaluate their effects in a slightly older cohort including, initially, with acute dosing.

The Cognitive Drug Research (CDR) battery has a large database of normative data, which indicates that working memory begins to decline in middle age (Wesnes, personal communication). The battery has been used in approximately 1400 clinical trials—many involving the cognitive and mood effects of nutraceutical interventions, including *P. ginseng* (Kennedy *et al.*, 2001a, 2001b; 2002). The current study therefore utilised the CDR battery in order to allow direct comparison with these studies. Because our previous study found positive effects of *P. quinquefolius* in healthy young adults on selected tasks form the Computerised Mental Performance Assessment (COMPASS) battery, these tasks were also included to gauge whether similar effects would be observed on the same tasks in a slightly older cohort. The use of both batteries allowed cross-platform comparison of the two assessment systems. The current study therefore specifically aimed to extend previous findings by assessing the cognitive effects of a single dose of *P. quinquefolius* (200 mg encapsulated Cereboost™) selected on the basis of previous findings (Scholey *et al.*, 2010) on aspects of working memory processes in a slightly older group of middle-aged (40–60 years) healthy volunteers. Specifically, we hypothesised that the administration of Cereboost™ would be associated with improved working memory performance in this population.

MATERIALS AND METHODS

Design

The investigation was a single centre, double-blind, placebo-controlled, balanced, two-period, crossover design study conducted at the Centre for Human Psychopharmacology, Swinburne University, Australia. The trial was registered on the Australian New Zealand Clinical Trials Registry as ACTRN12610000849099.

Participants

A power calculation produced an N of 46 to detect a significant change with a power of 0.8 at the 0.005 alpha levels (adjusted from 0.05 to allow for multiple comparisons). As a result, there was a requirement to recruit at least 50 participants to allow for dropout.

Fifty-four volunteers (22 male and 32 female volunteers, mean age 51.63, SD 6.35 years) took part in this study approved by the Swinburne University Human

Research Ethics Committee and conducted according to the Declaration of Helsinki. All participants gave written informed consent and received AU $200 for their participation upon the completion of the study.

Participants completed a health screening questionnaire, and all reported that they were in good health, not taking any drugs or medications (excluding the contraceptive pill), had no known food allergies and were non-smokers. Participants with a number of medical conditions such as diabetes, hypoglycaemia, psychiatric disorders, epilepsy and gastrointestinal disorders, who were taking prescribed medications, were pregnant or lactating were excluded from the study. They were screened for cognitive decline using the Mini-Mental State Examination (Folstein *et al.*, 1975), and all had scores of 27 and above, indicating that they were cognitively intact. Subjects also completed the Depression Anxiety and Stress Scale (Lovibond and Lovibond, 1995) to confirm absence of the three negative affective states of depression, anxiety and stress.

Enrolled participants refrained from taking any vitamins, other herbal supplements and over the counter medicines for the period of the study. On the testing days, participants also abstained from consuming alcohol, caffeine products and energy drinks.

Two participants withdrew from the study (one for reasons unrelated to the study and one due to an adverse event (AE) remotely related to the study product). These volunteers were excluded from analysis, giving a total sample size of 52 participants.

Treatments

Commercial extract of *P. quinquefolius* Cereboost™ provided by Naturex (Naturex, Avignon, France) was prepared according to methods described by Scholey *et al.* (2010). According to analysis of ginsenosides by high-performance liquid chromatography performed by a manufacturer, Cereboost™ was standardised to 11.65–11.67% ginsenosides with individual ginsenoside content as follows: Rb1 (5.69%), Re (2.06%), Rc (1.87%), Rd (1.48%), Rb2 (0.29%) and Rg1 (0.28%). Cellulose powder (Avicel® PH Microcrystalline Cellulose, FMC BioPolymer, PA) served as a placebo. Encapsulation was conducted at Thompsons Amcal Pharmacy in Melbourne, Australia. Treatments were soft, opaque, gelatine-based capsules of identical appearance, taste and smell. In order to adhere to double-blind methodology standards, each individual's treatments were prepared by a disinterested third party who took no further part in the study. On each of the two study days, participants received one capsule containing either 200 mg of Cereboost™ or placebo, depending on the condition to which they were

allocated on that particular day. Counterbalanced treatment allocation was performed by computer-generated randomisation.

Cognitive and mood measures

Cognitive Drug Research computerised assessment battery. The CDR computerised assessment battery has been used in more than 1400 drug and nutritional interventional trials. The tailored version of the CDR battery utilised here, including a description of the constituent tasks, has been described in detail previously (Scholey and Kennedy, 2004; Scholey *et al.*, 2008). This battery has previously been found to be sensitive to modulation of cognitive function as a consequence of acute ingestion of *P. ginseng* (Kennedy *et al.*, 2001b; 2002), Ginkgo biloba (Kennedy *et al.*, 2000; 2001a; 2002; 2007) and a Ginkgo biloba/ *P. ginseng* combination (Kennedy *et al.*, 2001a).

The selection of computer-controlled tasks was administered in fixed order with parallel versions of the tasks randomly presented between participants at each testing session. Presentation was via high-resolution computer monitors and, all responses were recorded via two-button (yes/no) response boxes with the exception of written word recall tests. The tasks were presented as follows: Word presentation, immediate word recall, picture presentation, simple reaction time, digit vigilance, choice reaction time, spatial working memory, numeric working memory, delayed word recall, word recognition and picture recognition. In all, CDR battery tasks took approximately 20 min to complete. Detailed description of the tasks can be found in previous publications (Kennedy *et al.*, 2000; 2001a; 2002; 2007; Pengelly *et al.*, 2012; Wesnes *et al.*, 2000; Sunram-Lea *et al.*, 2005).

As with previous studies assessing *P. ginseng*, the single task outcomes from the CDR battery were collapsed into the five cognitive outcome factors derived by a factor analysis as previously described by Wesnes *et al.* (2000) and presented graphically by Scholey and Kennedy (2004). The factor composition is briefly outlined in the following text.

'Working Memory' factor: derived by combining the sensitivity index scores from the two working memory tests—spatial working memory and numeric working memory. 100% accuracy across the two tasks would generate a maximum score of 2 on this index.
'Secondary Memory' factor: derived by combining the percentage accuracy scores from delayed word recognition, delayed picture recognition, immediate word recall and delayed word recall tasks. Accuracy

of 100% across the four tasks would generate a maximum score of 400 on this index.

'Speed of Memory' factor: derived by combining the reaction times of numeric working memory, spatial memory, delayed word recognition and delayed picture recognition (units are summed milliseconds from the four tasks).

'Speed of Attention' factor: 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).

'Accuracy of Attention' factor: derived by calculating the combined percentage accuracy across the choice reaction time and digit vigilance tasks. Accuracy of 100% across the two tasks would generate a maximum score of 100.

'Speed of Attention' and 'Accuracy of Attention' factors were described as 'Power of Attention' and 'Continuity of Attention' in the original factor analysis by Wesnes *et al.* (2000); however, description utilised in this research has been implemented in previous research on the effects of herbal extracts on cognition (e.g. Kennedy *et al.*, 2002; Sunram-Lea *et al.*, 2005; Haskell *et al.*, 2007).

Computerised Mental Performance Assessment System battery. The COMPASS testing battery has been developed to include tests, which have been shown to be sensitive to nutritional manipulations including to acute administration of American ginseng, and tasks are described in detail by Scholey *et al.* (2010). Parallel versions of each of the following tasks allowed for multiple testing. This suite of tasks took approximately 10 min to complete, and they were presented as follows:

Alphabetic working memory: A series of five letters were presented on the computer screen for 4 s for participants to memorise. This was followed by a series of 30 probe letters. Participants had to indicate whether the letter was from the original series pressing computer keyboard keys corresponding to YES or NO as quickly as possible. The task was scored for accuracy (%) and speed (ms) of performance.

Serial 7: Participants were required to count backwards in sevens from a random starting number between 800 and 999, presented on the computer screen, as quickly and accurately as possible. Participants were using the numeric key pad to enter each three-digit response. After the first subtraction was entered via the numeric key pad, each digit was replaced on the screen by an asterisk. The task was scored for a number of measures: total number of generated subtractions (n), number of correct (n) as well as incorrect (n) subtractions, average accuracy (%) and speed (ms) of performance, which evaluated average time taken participant to complete one subtraction (ms). In case of incorrect responses, the subsequent responses were scored as positive if they were correct in relation to the new number.

RVIP: A series of digits were presented on the computer screen, one at a time, at the rate of 100 digits per min in quick succession. Participants monitored continuous series for the target sequences: they were required to detect consecutive sequences of three even (combination of the following 2-4-6-8) or odd (combination of the following 1-3-5-7-9) numbers and asked to respond by pressing a space bar as soon as a target string was detected. Duration of the task was 3 min with eight correct target strings presented per minute. The task was scored for accuracy of performance (% of correctly identified target strings), the average reaction time (ms) to the correctly identified sequences, and the number (n) of false alarms.

Corsi Blocks: In all nine identical blue blocks (size 93 × 93 pixels) were presented dispersed randomly on the computer screen. A predetermined number of blocks were illuminated (briefly changed colour to red) sequentially at the rate of one per second identifying a sequence of spatial locations to be remembered. Volunteers repeated presented sequence by clicking boxes on the screen pointing cursor and clicking computer mouse. Task difficulty progressively increased increasing number of the presented blocks from four upwards (maximum of nine sequentially illuminated blocks). The task outcome (score) was a measure of spatial span, which reflected maximum of squares participants were able to repeat correctly.

Visual analogue mood scales: The computerised version of Bond–Lader Mood scales (Bond and Lader, 1974) was incorporated within a number of the selected COMPASS tasks. In all, 16 mood scales, comprised of 100 mm visual analogue scales anchored at each end by pairs of mood antonyms (e.g. calm-tense). These were combined as recommended by the authors to form three mood factors: 'alert', 'calm' and 'content'. Two other visual analogue scales assessed stress and mental fatigue and were described previously by Scholey *et al.* (2010). Briefly, participants were required to place a mark along the line of the visual analogue scale with end points anchored by 'not at all' and 'extremely' in response to the questions 'How (stressed/mentally fatigued) do you feel right now?' Scores ranged

from 1 to 100 with higher scores reflecting higher subjective feelings of stress/mental fatigue.

Pencil-and-paper questionnaires

Depression Anxiety and Stress Scales (DASS): The shortened 21-item version of the DASS (Lovibond and Lovibond, 1995) was used to assess three negative affective states of depression, anxiety and stress on seven-item scales on the practice day. The Depression subscale (DASS-D) measures symptoms relating to dysphoric mood (e.g. sadness), for example 'I couldn't seem to experience any positive feeling at all'. The Anxiety subscale (DASS-A) assesses symptoms associated with physiological hyperarousal such as autonomic arousal, for example 'I felt I was close to panic'. The Stress subscale (DASS-S) assesses symptoms associated with nervous arousal, for example 'I tended to over-react to situations'.

Mini-Mental State Examination is a standard tool for screening cognitive impairments (Folstein *et al.*, 1975). It is a brief 30-point questionnaire test, which facilitates the detection of mental status changes, especially in older cohorts, and frequently used to verify absence of cognitive decline (Folstein, 2007). The Mini-Mental State Examination was completed during the practice day as a part of screening procedure with a score equal or greater than 24 as an inclusion criterion.

Profile of Mood States (POMS) is a self-report questionnaire designed to measure six dimensions of mood: tension-anxiety; depression-dejection; anger-hostility; vigour-activity; fatigue-inertia; and confusion-bewilderment). The Profile of Mood States consists of 65 adjectives describing feeling and mood which is answered on a five-point Likert-type scale ranging from not at all to extremely. Respondents are asked to indicate mood reactions for the 'past week including today' or for shorter periods such as 'right now.' This questionnaire was completed at the beginning of the testing days to identify whether there were differences in participants' mood at the start of the day (before the baseline assessments and subsequently treatment administration), which could have potentially influenced the levels of cognitive performance during the day.

State-Trait Anxiety Inventory (STAI) comprised of two scales. The 'State' (STAI-S) subscale is widely used for measuring fluctuating levels of anxiety. The subscale contains 20 statements regarding current mood (e.g. 'I am calm'). Participants rate how much they feel like each statement at the time of making the response by marking a 4-point scale ranging from 'not at all' to 'very much so'. The 'Trait' (STAI-T)

subscale comprises 20 different statements (e.g. 'Some unimportant thought runs through my mind and bothers me'). Participants were asked to indicate how they generally feel on a scale ranging from 'almost never' to 'almost always'. Scores on both sections of the STAI range from 20 to 80, with higher scores indicating more anxiety. Participants completed this scale at the beginning of each visit to ensure that their anxiety levels did not differ prior to the commencement of the assessments and administration of treatments.

Leeds Sleep Evaluation Questionnaire (SEQ) comprises of 10 self-rating 100-mm-line analogue questions concerned with aspects of sleep and early morning behaviour. The questionnaire has been used to monitor subjectively perceived changes in sleep during psychopharmacological investigations. SEQ contains ten questions pertaining to four aspects of sleep: getting to sleep, quality of sleep, awakening from sleep, and behaviour following wakefulness. This questionnaire was completed at the beginning of each visit prior to the cognitive and mood assessments and administration of treatments to ensure that the quality of the previous night's sleep have not influenced participants' performance.

Blood glucose measures. As a part of the screening procedure, fasting blood glucose measurements were taken at the beginning of the practice day as well as each testing day prior to breakfast intake to ensure participants fasting blood glucose was <6.1 mmol/l. This value was chosen as the top end of a healthy fasting range as recommended by the local Diabetes Victoria (www.diabetesvic.org.au), also refer to Alberti and Zimmet (1998). On each of the two active study days, blood glucose levels were measured via capillary finger prick at a baseline (before a baseline assessment), then 1 h (before the commencement of the first post-treatment assessment), 3 h (before the commencement of the second post-treatment assessment) and 6 h (before the commencement of the third) post-treatment assessment following treatment administration. Blood glucose levels were monitored using a MediSense Optium Xceed Blood Glucose Sensor and disposable MediSense Blood Glucose Electrodes (MediSense Britain Ltd, Birmingham, UK). The accuracy and consistency of MediSense blood glucose sensors has previously been established (e.g. (Matthews *et al.*, 1987). The reliability of the test has previously been confirmed (Price and Koller, 1988). Blood samples were taken using Owen Mumford 'Unistik 2' single use capillary blood sampling devices (Owen Mumford Ltd, Oxford, UK). Alcohol-soaked skin cleansing swabs was used for pre-sampling sterilisation.

Blood pressure and heart rate measurement. Participants were seated calmly in a comfortable relaxed position for at least 10 min prior to the procedure. Blood pressure was then measured with an automated blood pressure sphygmomanometer, making sure that an arm with a fastened blood pressure cuff was at participants' heart level. Systolic blood pressure was expected to be between 91 and 139 mmHg and diastolic blood pressure between 61 and 89 mmHg and only participants whose blood pressure lay within normal ranges were included in the study. Heart rate was also recorded; this measure was not used for screening purposes.

Tolerability. Tolerability was assessed via monitoring of unsolicited AEs ('How have you been feeling'). To elicit reports of adverse events and inter-current illnesses, participants were required to complete the Symptom Checklist questionnaires at the beginning (Weekly Symptom checklist) and the end of each test session (Daily Symptom checklist). The symptom checklist was developed at the Brain Sciences Institute specifically for use with natural medicines and consists of 28 physiological/psychological problems people might have, for example, 'I feel dizzy', 'I have a dry mouth' and 'I feel anxious more than usual' (Stough *et al.*, 2001). Participants indicated how much the problem had bothered them in the last 7 days including the day of recording using a 5-point scale from 'not at all' to 'very much so'.

Procedure

Each participant was required to attend the testing labs on a total of three occasions (one practice and two study days), conducted seven days apart to ensure sufficient wash-out between conditions. Testing took place in a suite of dedicated laboratories at the Centre for Human Psychopharmacology with participants visually isolated from each other. Participants were also wearing headphones to minimise distraction.

At the beginning of the first visit, participants signed their informed consent and then underwent a health screen procedure during which their morphometric and demographic data were collected and blood pressure, heart rate and fasting blood glucose were measured. Based on the outcomes of the health screen, participants eligible for the trial were familiarised with the protocol and procedures of the study. Additionally, participants filled in pen-and-paper questionnaires and completed three cycles of the computerised tasks of CDR and COMPASS batteries to minimise practice effects. Performance data from the practice session were not included in the analysis.

On arrival at their first testing visit, participants were randomly allocated to the treatment order determined by a computer-generated matrix. Fasting blood glucose was measured at the beginning of each visit to ensure that participants fasted before attending sessions, which was followed by standard light breakfast (piece of toast or cereal) consumed at least 1 h before the onset of the experiment. During this hour, prior to baseline assessment, they completed selected pen-and-paper questionnaires (STAI-S, POMS and Leeds SEQ) and later underwent four identical cognitive and mood assessments using parallel versions of the CDR battery as well as parallel versions of selected tasks from the COMPASS battery. Entire selection of the computer tasks (CDR and COMPASS) took approximately 30 min to complete. The first was a pre-dose testing session, which established baseline performance for the day, was immediately followed by the day's treatment (*P. quinquefolius* or placebo). Further testing sessions began at 1, 3 and 6 h following the consumption of the treatment. A standard light lunch was provided upon completion of 1 h post-dose assessment (between 1 and 3 h cognitive and mood assessments or approximately 1.5 h post-dose), which consisted of sandwich (with either chicken and salad, or cheese and salad), the same foods were consumed by each participant on each study day. Each assessment was preceded by blood glucose measurements (see Blood glucose measurements for details). The symptom checklist was completed at the end of the final testing session of the day to make sure that it was safe for participants to leave testing laboratories.

Statistical analysis

Only the data of the participants who attended all three visits and had a suitable level of compliance with all

Table 1. Demographic and morphometric characteristics of the volunteers (N = 52)

Measure	Mean	SD	Min	Max
Age (years)	51.63	6.47	40	60
Education (years)	16.60	3.04	11	25
Height (cm)	169.65	10.94	147	196
Body weight (kg)	71.15	14.23	45	110
Body mass index (kg/m^2)	24.5	2.9	18.7	30
Systolic blood pressure (mmHg)	126.00	11.36	99	139
Diastolic blood pressure (mmHg)	78.2	8.5	63	89
Heart rate (bpm)	68.98	10.94	51	99
Fasting blood glucose (mmol/L)	5.04	0.52	4.1	6.00
MMSE (score)	29.23	0.81	27	30
DASS (score)	27.35	4.26	21	39
STAI-trait (score)	35.85	9.34	21	47

MMSE, Mini-Mental State Examination; DASS, Depression Anxiety and Stress Scales; STAI, State-Trait Anxiety Inventory.
Mean scores are presented with standard deviations and ranges.

Table 2.   Effects of *P. quinquefolius* (Cereboost[TM]) on individual task outcome measures from the Cognitive Drug Research battery

Measure	(n)	Pre-dose baseline score	Post-dose change from baseline score		
			1 h	3 h	6 h
Immediate word recall accuracy (%)					
Placebo	51	41.69 (13.65)	−1.96 (13.81)	−4.05 (14.43)	−1.57 (14.88)
P. quinquefolius		44.05 (11.78)	−6.80 (11.74)	−5.49 (13.04)	−7.97 (13.92)
Simple reaction time (ms)					
Placebo	49	266.83 (42.36)	16.17 (27.12)	11.15 (30.53)	11.05 (23.47)
P. quinquefolius		269.68 (45.01)	14.25 (25.64)	12.03 (21.76)	13.01 (27.84)
Digit vigilance accuracy (%)					
Placebo	43	98.35 (1.95)	−0.93 (5.21)	−1.03 (4.45)	−0.26 (3.15)
P. quinquefolius		98.38 (2.23)	0.26 (2.23)	−0.36 (3.29)	−0.77 (3.21)
Digit vigilance FA (n)					
Placebo	50	1.03 (1.05)	−0.54 (1.25)	−0.34(1.22)	−0.40 (1.39)*
P. quinquefolius		0.76 (0.73)	−0.18 (1.00)	0.00 (1.11)	0.12 (1.33)
Digit vigilance RT (ms)					
Placebo	50	413.60 (40.59)	6.78 (25.16)	2.18 (31.36)	8.27 (33.76)
P. quinquefolius		413.92 (40.13)	3.63 (28.45)	9.58 (26.83)	6.61 (27.13)
Choice RT accuracy (%)					
Placebo	50	98.03 (1.97)	0.44 (2.07)	−0.36 (2.70)	−0.40 (2.46)
P. quinquefolius		97.80 (1.84)	−0.04 (2.66)	0.28 (2.52)	0.08 (2.99)
Choice RT (ms)					
Placebo	49	454.80 (46.77)	12.29 (49.42)	6.02 (45.33)	16.37 (53.21)
P. quinquefolius		458.90 (68.68)	4.31 (36.22)	4.20 (41.52)	8.02 (40.38)
Spatial WM (SI)					
Placebo	44	0.96 (0.05)	−0.05 (0.14)	**−0.15 (0.35)***	−0.09 (0.19)
P. quinquefolius		0.93 (0.09)	−0.10(0.29)	**−0.01 (0.15)**	−0.02 (0.18)
Spatial WM RT (ms)					
Placebo	49	839.65 (210.58)	39.98 (243.36)	10.65 (270.00)	94.52 (367.73)
P. quinquefolius		865.66 (216.99)	2.73 (212.47)	17.48 (206.65)	34.78 (350.89)
Numeric WM (SI)					
Placebo	46	0.95 (0.04)	−0.02 (0.08)	−0.03 (0.08)	−0.01 (0.09)
P. quinquefolius		0.95 (0.05)	0.01 (0.07)	−0.00 (0.06)	0.00 (0.07)
Numeric WM RT (ms)					
Placebo	49	692.85 (130.85)	−7.99 (79.82)	−15.45 (83.73)	−17.90 (98.73)
P. quinquefolius		680.78 (115.17)	−10.13 (67.51)	−28.34 (61.31)	−15.57 (73.60)
Delayed word recall accuracy (%)					
Placebo	50	30.06 (15.74)	−8.13 (15.20)	−12.00 (16.77)	−10.13 (15.84)
P. quinquefolius		30.19 (12.17)	−10.40 (11.12)	−9.87 (12.51)	−11.07 (12.53)
Word recognition (SI)					
Placebo	50	0.63 (0.18)	−0.00 (0.18)	−0.09 (0.23)	−0.05 (0.16)
P. quinquefolius		0.65 (0.17)	−0.07 (0.23)	−0.07 (0.22)	−0.09 (0.20)
Word recognition RT (ms)					
Placebo	50	848.01 (179.71)	12.54 (152.68)	6.41 (166.92)	−8.16 (169.70)
P. quinquefolius		826.36 (169.01)	14.04 (115.92)	20.78 (118.40)	53.20 (180.00)
Picture recognition (SI)					
Placebo	49	0.75 (0.18)	−0.04 (0.16)	−0.03 (0.20)	−0.07 (0.17)
P. quinquefolius		0.75 (0.21)	0.00 (0.18)	−0.04 (0.17)	−0.05 (0.16)
Picture recognition RT (ms)					
Placebo	49	1006.33 (215.35)	−23.29 (170.33)	−6.67 (162.80)	−27.17 (185.22)
P. quinquefolius		989.55 (230.05)	13.69 (240.65)	−0.65 (171.72)	39.96 (243.87)

Mean baseline and change from baseline scores are presented with standard deviations in parentheses. *p*- values between *P. quinquefolius* and placebo with Bonferroni correction are indicated (*, <0.05).

study protocol requirements were selected for analyses. Across the participants, screening for univariate outliers revealed that scores for each outcome measure were normally distributed. Data points with mean scores more than three standard deviations outside the sample means were excluded from the analyses; therefore, a number of participants vary between the tasks depending on their performance on particular

Table 3. Effects of *P. quinquefolius* (Cereboost[TM]) on cognitive factors derived from the Cognitive Drug Research battery

Measure	(n)	Pre-dose baseline score	Post-dose change from baseline score		
			1 h	3 h	6 h
Speed of attention (summed ms)					
Placebo	48	1127.92 (100.03)	33.18 (65.38)	−17.13 (54.51)	17.66 (74.49)
P. quinquefolius		1142.51 (133.95)	21.69 (60.94)	4.61 (49.54)	−0.88 (49.37)
Accuracy of attention (%)					
Placebo	43	92.35 (1.80)	0.23 (2.92)	−0.70 (3.41)	0.51 (2.68)
P. quinquefolius		92.45 (1.34)	0.42 (1.65)	−0.35 (2.64)	−0.16 (1.98)
Speed of memory (summed ms)					
Placebo	48	3370.98 (541.35)	28.74 (322.65)	15.17 (366.38)	45.24 (485.60)
P. quinquefolius		3349.90 (545.73)	12.84 (353.33)	9.15 (301.62)	106.50 (613.02)
Secondary memory (% X 4)					
Placebo	48	199.59 (50.16)	−20.35 (40.51)	−32.81 (48.42)	−26.94 (51.15)
P. quinquefolius		201.99 (50.38)	−28.61 (36.92)	−29.06 (42.09)	−34.06 (44.07)
Working memory (SI X 2)					
Placebo	40	1.92 (0.08)	−0.07 (0.19)	**−0.16 (0.37)***	−0.10 (0.23)
P. quinquefolius		1.88 (0.11)	−0.11 (0.29)	**−0.02 (0.12)**	−0.03 (0.20)

Mean baseline and change from baseline scores are presented with standard deviations in parentheses. *p*-values between *P. quinquefolius* and placebo with Bonferroni correction are indicated (*, <0.05)
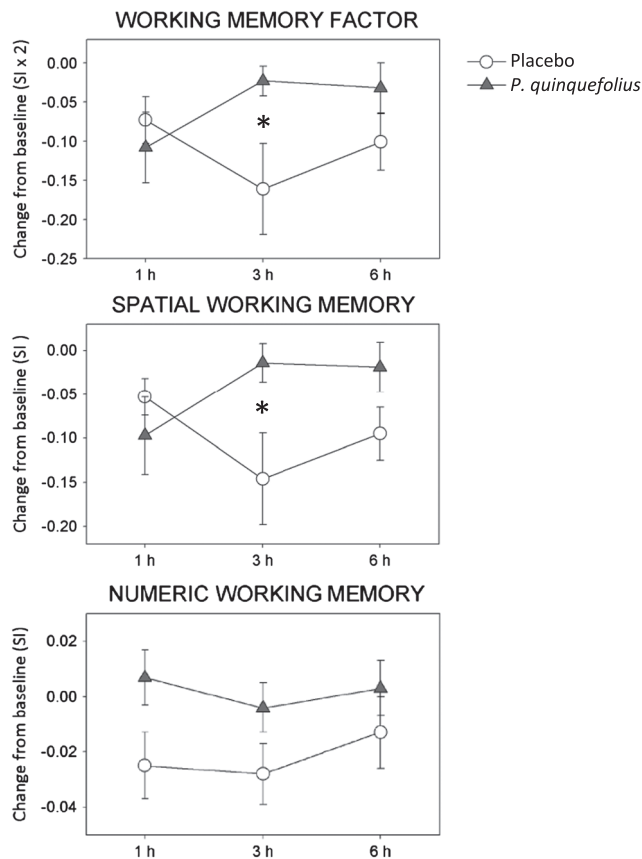

Figure 1. Effects of *P. quinquefolius* (Cereboost[TM]) on working memory measures. Graphs depict mean scores with SEM 1, 3 and 6 h following a single dose of *P. quinquefolius* and placebo. Significant differences (*, <0.05) at each time point are indicated

measure. This especially evident in relation to Factor scores, which are composite scores based on performance not one but a number of tasks; however, it was confirmed that the treatment order was not affected by the removal of the outliers.

Scores from the pencil-and-paper questionnaires (POMS, Leeds SEQ and STAI-S) administered prior to the baseline assessment to ensure there were no in participant emotional and physical state between 2 days prior to treatment administration, which could affect cognitive performance and mood, were analysed with a one-way repeated-measures analysis of variance (ANOVA).

Scores on all CDR individual task outcomes, the five primary factors, the selected tasks from COMPASS battery outcomes and blood glucose levels were analysed as 'change from baseline' using the SPSS 17.0 statistical package for Windows. Prior to analysis of 'change from baseline' data, raw baseline scores for all these measures were subjected to a one-way repeated-measures ANOVA comparing placebo with *P. quinquefolius* to ensure there were no differences of performance between the study days. Next step, change-from-baseline scores were subjected to a two-way repeated-measures ANOVA with factors being 'treatment' (Cereboost[TM], placebo) and 'time' (1, 3 and 6 h) with repeated measures on both factors. For this initial ANOVA, all testing was two-tailed at 5% significance level. Bonferroni corrected paired contrasts were made comparing *P. quinquefolius* with

Table 4.   Effects of *P. quinquefolius* (Cereboost[TM]) on individual task outcome measures from the Computerised Mental Performance Assessment battery

Measure	(n)	Pre-dose baseline score	Post-dose change from baseline score		
			1 h	3 h	6 h
Corsi Blocks score					
Placebo	52	4.87 (1.95)	0.10 (1.45)	0.13 (1.55)	0.22 (1.52)
P. quinquefolius		5.15 (1.91)	−0.05 (1.39)	−0.24 (1.28)	0.06 (1.45)
Serial 7s accuracy (%)					
Placebo	51	85.55 (13.23)	−1.51 (14.77)	−0.24 (14.05)	3.10 (12.65)
P. quinquefolius		84.35 (14.64)	1.37 (17.10)	3.14 (12.15)	4.95 (13.39)
Serial 7s presented (n)					
Placebo	52	22.94 (12.11)	2.10 (5.21)	2.79 (4.27)	2.33 (4.70)
P. quinquefolius		23.00 (11.33)	1.10 (4.97)	3.13 (5.60)	2.63 (5.52)
Serial 7s correct (n)					
Placebo	52	20.34 (12.27)	1.67 (5.96)	2.79 (5.03)	2.75 (5.59)
P. quinquefolius		19.88 (11.41)	1.25 (6.48)	3.63 (6.02)	3.27 (5.83)
Serial 7s incorrect (n)					
Placebo	52	2.56 (1.90)	0.46 (2.48)	0.02 (2.19)	−0.40 (2.29)
P. quinquefolius		3.09 (2.26)	−0.54 (2.77)	−0.50 (2.34)	−0.63 (2.41)
Serial 7s speed (ms)					
Placebo	52	6541.51 (3457.35)	−126.71 (2517.08)	−232.58 (2270.72)	−587.50 (1806.76)
P. quinquefolius		6381.09 (3303.72)	−136.50 (2756.68)	−733.02 (2051.93)	−530.36 (2953.07)
AWM accuracy (%)					
Placebo	49	94.51 (4.19)	1.20 (3.62)	0.43 (4.38)	0.88 (4.06)
P. quinquefolius		94.13 (4.85)	0.57 (4.55)	1.09 (4.61)	0.84 (4.74)
AWM speed (ms)					
Placebo	49	568.87 (156.17)	−33.69 (96.30)	−48.18 (96.12)	−53.77 (83.55)
P. quinquefolius		559.55 (147.14)	−8.88 (84.49)	−18.22 (106.10)	−29.50 (93.35)
RVIP accuracy (%)					
Placebo	50	45.12 (28.53)	0.96 (31.31)	−2.31 (32.63)	6.62 (28.76)
P. quinquefolius		48.07 (25.62)	−2.92 (24.11)	−2.48 (22.57)	2.10 (21.17)
RVIP FA (n)					
Placebo	49	8.04 (7.23)	0.67 (10.26)	4.45 (18.58)	−0.88 (8.03)
P. quinquefolius		10.46 (9.19)	−0.45 (11.30)	−0.84 (9.88)	0.18 (22.89)
RVIP speed (ms)					
Placebo	48	497.68 (74.67)	−15.35 (59.78)	−16.74 (58.31)	−9.01 (66.65)
P. quinquefolius		485.47 (60.74)	−9.06 (63.44)	−8.46 (56.57)	−8.65 (66.88)

Mean baseline and change from baseline scores are presented with standard deviations in parentheses.

placebo at each time point. To ensure the overall Type I error protection level, only comparisons associated with measures that generated a significant main effect of Treatment, or Treatment × Time interaction on the initial ANOVA are reported, along with associated significant and trend differences between treatments at each time point.

RESULTS

Participant characteristics

Demographic and morphometric characteristics of the participants are summarised in Table 1. Of the 52 participants who completed the trial 22 (42.3%) were male, 44 (84.6%) were right-handed and eight (15.4%), 24 (46.2%) and 20 (38.5%) had secondary, tertiary and postgraduate education, respectively.

Baseline scores

According to a one-way repeated-measures ANOVA analysis of all computerised and pencil-and-paper scores comparing placebo with *P. quinquefolius,* there were no significant differences on any measure between the two conditions prior to treatment administration.

Cognitive Drug Research individual tasks outcome measures

Mean pre-dose baseline raw scores and change from baseline scores, for each treatment at each post-time point on the individual tasks are presented in Table 2. Where appropriate, the results of the planned comparison of individual task outcomes that generated a significant result on the initial ANOVA are described in the succeeding text in relation to the overall factor to which they contribute.

Cognitive factors

Mean raw baseline scores and change from baseline scores for each condition across each session are presented in the table and graphs of Table 3 and Figure 1.

Table 5. Effects of *P. quinquefolius* (Cereboost™) on the mood factors derived from the Bond–Lader mood scales (Alert, Content and Calm) and on the 'mental fatigue' and 'stress' Visual Analogue Scales mood scores

Measure	(n)	Pre-dose baseline score	Post-dose change from baseline score		
			1 h	3 h	6 h
Mood factor alert					
Placebo	50	66.02 (15.23)	−6.75 (14.59)	−9.74 (16.13)	−12.83 (17.12)
P. quinquefolius		67.85 (13.38)	−7.62 (14.93)	−8.11 (14.15)	−12.31 (17.10)
Mood factor calm					
Placebo	50	65.81 (16.02)	−3.06 (12.55)	−3.49 (14.38)	−2.71 (12.48)
P. quinquefolius		66.67 (16.75)	−2.27 (9.50)	−4.06 (11.56)	−4.61 (13.82)
Mood factor content					
Placebo	50	72.58 (13.24)	−2.45 (10.96)	−3.19 (11.38)	−6.12 (10.43)
P. quinquefolius		73.38 (14.54)	−2.86 (9.85)	−3.37 (10.34)	−4.08 (10.62)
VAS stress (mm)					
Placebo	51	43.86 (18.95)	7.88 (16.00)	8.88 (18.35)	14.43 (23.00)
P. quinquefolius		42.42 (22.81)	6.92 (20.45)	9.65 (20.11)	12.82 (22.27)
VAS fatigue (mm)					
Placebo	51	40.71 (20.34)	4.94 (19.84)	4.47 (21.07)	1.35 (19.25)
P. quinquefolius		41.63 (23.85)	−0.37 (18.51)	1.65 (21.57)	1.90 (21.08)

Mean baseline and change from baseline scores are presented with standard deviations in parentheses.

Working memory factor: The working memory factor was the primary outcome. The initial ANOVA showed a significant treatment x time interaction [$F(2,78)=3.380$, $p=0.039$]. Compared with placebo, performance on this factor was significantly improved by Cereboost™ at 3 h ($p=0.017$) with a trend towards improved performance at 6 h ($p=0.08$) post treatment (Table 3 and Figure 1).

Inspection of the individual task outcomes contribution to the working memory factor showed that there was a significant Treatment×Time interaction [$F(2,86)=3.891$, $p=0.024$] on the initial ANOVA (Table 2 and Figure 1) for Spatial Working Memory sensitivity index. Compared with placebo, Cereboost™ was associated with an improvement on this task at 3 h ($p=0.022$) with a trend towards a significant improvement at 6 h post treatment ($p=0.058$).

There was no significant Treatment or Treatment×Time interactions for the secondary memory, speed of memory, accuracy of attention or speed of attention factors.

COMPASS individual tasks outcome measures

Mean pre-dose baseline raw scores and change from baseline scores, for each treatment at each post-time point on the selected tasks of the COMPASS battery are presented in Table 4. There were no significant main effects of treatment or any Treatment×Time interactions for any COMPASS measure.

Mood measures

None of the mood measures were significantly affected by treatment (Table 5).

Blood glucose levels

According to initial ANOVA [$F(1,52)=0.088$, $p=0.768$], blood glucose levels were not affected by the treatment and there were no treatment x time interactions (Table 6).

Table 6. Effects of *P. quinquefolius* (Cereboost™) on peripheral levels of blood glucose

Measure	(n)	Pre-dose baseline score	Post-dose change from baseline score		
			1 h	3 h	6 h
Blood glucose levels (mmol/litre)					
Placebo	52	6.56 (1.32)	−1.73 (1.58)	−0.31 (1.67)	−2.04 (1.37)
P. quinquefolius		6.54 (1.19)	−1.65 (1.26)	−0.33 (1.28)	−1.87 (1.71)

Mean baseline and change from baseline scores are presented with standard deviations in parentheses.

Tolerability

The product was well tolerated. One AE was recorded during the study, and was classified as remotely related to the study product.

DISCUSSION

This randomised, crossover, placebo-controlled study investigated the acute cognitive, mood and glucoregulatory effects of a single (200 mg) dose of *P. quinquefolius* (Cereboost™) in middle-aged (40–60 years) volunteers. Supporting our hypothesis, Cereboost™ improved working memory performance as indexed by the working memory factor score of the CDR test battery. This effect was time dependent, with significant improvement observed at 3 h and a trend towards improvement at 6 h post treatment. Whilst a working memory factor encompassed accuracy indices (SI) of both spatial and numeric working memory, improvement was largely driven contributed by the spatial working memory task (Figure 1). The current study demonstrated that a single dose of 200 mg of Cereboost™ elicited modulation of cognitive performance of healthy middle-aged adults in the absence of mood effects.

These results partially replicate previously reported effects of Cereboost™ on cognitive functions in a younger population, with treatment-related improvements mainly observed within the cluster of the tasks targeting working memory (Scholey *et al.*, 2010). The previous randomised, double-blind, placebo-controlled, crossover trial examined the acute mood, neurocognitive and glycaemic effects of three doses (100, 200 and 400 mg) of *P. quinquefolius* in healthy young adults (mean age = 25.2 years, ±4.97). Like here, there was a significant improvement of working memory performance associated with *P. quinquefolius.* The middle 200 mg dose differentially improved speed of numeric working memory. Both the lower (100 mg) and the higher (400 mg) doses enhanced alphabetic working memory speed at all time points. In addition, all three doses significantly improved Corsi blocks performance at all testing time points. Counter to predictions, the 200 mg of *P. quinquefolius* did not improve performance of Corsi blocks and alphabetic working memory in the older middle-aged cohort. However, differences in populations (middle-aged vs. young adults) may be partially accountable for the observed differences. Another possible explanation could be the structure of the testing days. In the current study, participants attended the laboratory for approximately 8.5 h, almost 2 h longer than the previous

study. Extending length of the study may have induced fatigue. The Corsi block tasks came relatively late in a longer testing session than in the previous study in the young cohort. It is possible that participants expended cognitive and/or attentional resources on other tasks leading to a null effect on Corsi blocks.

The relatively high levels of ginsenoside Rb_1 in Cereboost™ *P. quinquefolius* may be partially responsible for its effect on cognition. According to Hasegawa (2004) and Tawab *et al.* (2003) ginsenosides, panaxadiols, and Rb_1 in particular, can be quickly absorbed from the gastrointestinal system and remain in the parent form after entering systemic circulation for hours. However, these compounds may also undergo a process of stepwise glycosylation facilitated by intestinal flora reaching systemic circulation as their degradation products (compound-K (M1), M4, Rh_1 and F1), where they are esterified to fatty acid conjugates remaining active longer than the parent compound. Indeed, it has been demonstrated that intestinal bacterial metabolic activity varies amongst individuals, and activity of orally administered ginsenosides correlates with the ginsenoside-hydrolyzing potential of intestinal flora (Lee *et al.*, 2009). As the deglycosylation process is crucial for pharmacological expression of ginsenosides, individual differences in bacterial ginsenoside-hydrolysing potential and gastric efficiency determined by various conditions including diet, health and stress levels may lead to different levels of ginseng efficacy (Hasegawa, 2004). Hence, it is possible that gastrointestinal efficiency of the middle-aged group or this particular group of volunteers differed from that of the participants recruited for the previous study, which determined differences in effects of ginsenosides on cognitive function and mood.

Choice of the single dose (200 mg) for investigating cognitive and mood effects of Cereboost™ in middle-aged cohort might have been another reason for discrepancies between the findings of the two studies. Some of the cognitive measures, such as choice reaction time and alphabetic working memory speed, were improved by the lowest (100 mg) and the highest (400 mg) doses in the younger cohort; therefore, assessment of these doses might have revealed a greater scope of benefits for cognition in middle-aged population. Therefore, implementation of the different dosages of the extract is required to further understanding of its effects in middle-aged adults.

Findings of the current study established that the ingestion of single 200 mg dose of Cereboost™ does not modulate blood glucose in middle-aged healthy

volunteers, with this pattern being consistent with outcomes of the preceding study, which investigated glucoregulatory effects of the same extract in a younger (18–40 years) age group (Scholey *et al.*, 2010). This is despite the fact that the cohort was older than in our previous study (Scholey *et al.*, 2010). This suggests that *P. quinquefolius* may not acutely modulate blood glucose levels despite the fact that the study was strictly controlled for glycaemic parameters. As there were no changes in blood glucose in either study, it seems unlikely that *P. quinquefolius* exerts mediating effects on cognitive function through glucose or insulin-mediated mechanisms.

Vuksan *et al.* (2000b) previously reported that *P. quinquefolius* root extract attenuated postprandial blood glucose when co-administered with 25 g of glucose in both healthy and diabetic volunteers; however, hypoglycaemic effects somehow differed between two groups. Prominent hypoglycaemic effects of *P. quinquefolius* were observed in diabetic population regardless of a dose of the herb or a time of its administration in relation to the glucose load. In contrast, a different profile of treatment-related activity emerged in healthy volunteers: it was effective when administered 40 min prior to a glucose challenge and in fasting conditions only. In relation to the current study, participants were required to fast prior to the sessions in order to control for consumption of standard breakfast in both treatment conditions, they were provided with food; therefore, implementation of fasting regiments may reveal a completely different pattern of glucoregulatory changes in response to *P. quinquefolius*.

Whilst the majority of studies have focused on the acute postprandial effects of *P. quinquefolius* alone or when administered in conjunction with a glucose load, other studies have attempted to delineate chronic effects of the herb in humans (Vuksan *et al.*, 2001; Vuksan and Sievenpiper, 2005). Administration of *P. quinquefolius* (1 g) three times daily (40 min before meals) to 24 subjects diagnosed with Type 2 Diabetes mellitus for 8 weeks resulted in significant reduction of fasting blood glucose and drop in levels of HbA1c accompanied by observable but insignificant increase of insulin levels suggestive of improved pancreatic β-cell function. Vuksan *et al.* (2001) offered an explanation that *P. quinquefolius* glucoregulatory efficacy may be related to modulation of the post-absorptive early phase of insulin secretion, the loss of which marks Type 2 Diabetes mellitus. Noteworthy, although *P. quinquefolius* generally tends to lower postprandial blood glucose, its extract with depressed ginsenoside profile was not effective (Sievenpiper *et al.*, 2004),

highlighting the significance of ginsenoside expression in *P. quinquefolius* extracts for glucoregulation. The outcomes of another study into the glucoregulatory effects of different ginseng types by Sievenpiper *et al.* (2004) indicated that the ratio of panaxadiols/panaxatriols is inversely correlated with glucose-lowering efficacy of ginsengs, which may explain differential potency of various extracts. Investigation of the glucoregulatory effects of Cereboost™ in populations with glucoregulatory deficiencies and in chronic regiments may help to specify its glucoregulatory properties.

The underlying neurocognitive mechanisms underpinning the effects of *P. quinquefolius* on working memory in healthy young and middle-aged adults remain to be established. To date, no published studies have investigated the effects of *P. quinquefolius* on brain bioactivity. However, Kennedy *et al.* (2003) reported acute effects of standardised extract of *P. ginseng* (G115) on brain electrical activity in 15 healthy young (mean age 26.6) volunteers. This double-blind, placebo-controlled, balanced crossover study implemented a standard auditory oddball paradigm when participants were paying attention to the infrequent target tones presented randomly amongst frequent non-target tones. Administration of *P. ginseng* (200 mg) initiated significant reduction of P300 latency of the auditory evoked response potential across left temporal and occipital regions without exerting changes in its amplitude. Additionally, *P. ginseng* modulated cerebral EEG activity registered in 'eyes closed' condition across a number of frequency bands. *P. ginseng* facilitated overall reduction in theta, beta and alpha frequency predominantly observed at the frontal sites. Authors suggested that these effects may be associated with ability of ginsenosides to modulate EEG activity through cholinergic neurotransmitter systems or their ability to increase cerebral blood flow hence intensify delivery of metabolic substrates to cerebral structures. Whilst the study provides evidence that *P. ginseng* facilitates measurable neurocognitive changes in humans, these results are unlikely to be pertinent to the current investigation of *P. quinquefolius,* as the profile of ginsenosides in Cereboost™ differs from that of *P. ginseng* extract G115 with respect to individual ginsenoside content, concentrations and the ratio of panaxadiols and panaxatriols. For example, levels of Rb1, Rd and Re ginsenosides in Cereboost™ is comparatively higher than that of G115 therefore pharmacological profiles of the extracts are likely to differ. Differences between the acute behavioural effects of *P. quinquefolius* and *P. ginseng* are slowly emerging facilitated by the

availability of the standardised extracts Cereboost™ and G115 and become evident from the broad evaluation of the studies based on robust double-blind, repeated-measures methodology with the use of the computerised cognitive and mood assessments.

Behavioural effects of *P. quinquefolius* reported by Scholey *et al.* (2010) and observed in the current investigation differ from the acute effects of *P. ginseng* (G115) outlined in the behavioural studies of Kennedy *et al.* (2001b; 2002). Briefly, *P. ginseng* was also capable of enhancing memory performance at levels that are comparable with pharmaceutical cognitive enhancers (Neale *et al.*, 2013). Improvements were largely associated, however, with episodic or 'secondary' memory with changes occurring up to 6 h post-dose in response to the intake of 400 mg dose (Kennedy *et al.*, 2001b; 2002). In contrast, the current research suggests that cognitive effects of *P. quinquefolius* are differentially associated with working memory processes.

The mechanisms behind facilitation of cognitive performance by Cereboost™ in both young and middle-aged volunteers are unlikely to be associated with a single physiological process. Central cholinergic systems have been implicated in mediating learning and memory processes (Perry, 1986) and cholinergic effects of isolated ginsenosides have been revealed in animal models. For example, Rb_1 increased expression of choline acetyltransferase and nerve growth factor messenger RNA (Salim *et al.*, 1997), stimulated synaptosomal choline uptake and acetylcholine release (Benishin *et al.*, 1991; Benishin, 1992) and modulated long-term potentiation in the rat hippocampal formation (Abe *et al.*, 1994). Results of several animal studies show that Rb_1 (Benishin *et al.*, 1991), Rg1 (Yamaguchi *et al.*, 1995) and Re (Yamaguchi *et al.*, 1996) prevent scopolamine-induced memory deficits, by increasing cholinergic activity. Future studies should elucidate the mechanisms of the observed effects of Cereboost™ on working memory via, for instance, scopolamine challenge in humans.

Furthermore, we cannot rule out the possibility of ginsenoside effects on other neurotransmitter systems (Kennedy and Scholey, 2003). Because cognitive improvements are most consistently found in working memory measures, one plausible explanation could be ginsenoside-mediated augmentation of the dopaminergic neurotransmitter system (Ellis and Nathan, 2001). On the other hand, considering evidence of the cholinergic contribution to these processes (Rusted and Warburton, 1989), and a relatively high content of ginsenoside Rb_1, possessing potent cholinergic effects (Benishin *et al.*, 1991) in Cereboost™ *P. quinquefolius*, interaction of ginsenosides with cholinergic systems may contribute to the observed cognitive effects.

In addition, capacity of ginsenosides to modulate nitric oxide production has been previously demonstrated (Smith *et al.*, 2014). Considering that nitric oxide enhances endothelial function and modulates cerebral blood flow, efficient delivery of metabolic substrates to the active cortical sites could be one of the potential mechanisms underlying cognitive improvements exerted by ginseng species. Additional measurements of cardiovascular function such as registration of cerebral blood flow of the participants in Cereboost™ and placebo conditions may provide useful insight into these mechanisms.

In summary, the present results confirm that Cereboost™ has the ability to positively modulate working memory in middle-aged adults, predominantly 3 h following administration. These results are broadly consistent with previous work in younger adults (Scholey *et al.*, 2010) where working memory was also improved. The mechanism of action remains unknown, but it is unlikely that regulation of blood glucose by *P. quinquefolius* is involved. Further research is required in order to evaluate the effects of Cereboost™ at different dosages in this and other populations.

CONFLICT OF INTEREST

ACKNOWLEDGEMENTS

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