REVIEW ARTICLE

The Safety and Efficacy of Botanicals with Nootropic Effects

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Abstract: Recent estimates for the global brain health supplement category, *i.e.* nootropic market size, will grow to nearly $5.8 billion by 2023. Overall, nearly one-quarter (23%) of adults currently take a supplement to maintain or improve brain health or delay and reverse dementia. Not surprisingly, the use of such supplements increases with age - more than one-third of the oldest generation (ages 74 and older) takes a supplement for brain health. This widespread use is being driven by a strong desire both in the younger and older generations to enhance cognitive performance and achieve healthy aging. The most prevalent botanicals currently dominating the nootropic marketplace include *Gingko biloba*, American ginseng, and *Bacopa monnieri*. However, other botanicals that affect stress, focus, attention, and sleep have also been procured by dietary supplement companies developing products for improving both, short and long-term brain health. This review focuses on efficacy data for neuroactive botanicals targeted at improving cognitive function, stress reduction, memory, mood, attention, concentration, focus, and alertness, including *Bacopa monnieri*, *Ginkgo biloba*, Holy basil, American ginseng, Gotu kola, Lemon balm, Common and Spanish sages and spearmint. Botanicals are discussed in terms of available clinical efficacy data and current safety profiles. Data gaps are highlighted for both efficacy and safety to bring attention to unmet needs and future research.

1. INTRODUCTION

Dietary supplements are defined by the United States (US) Food and Drug Administration (FDA) as "products taken by mouth that contain a dietary ingredient. Dietary ingredients include vitamins, minerals, amino acids, and herbs or botanicals, as well as other substances that can be used to supplement the diet," [1]. Supplements come in many formulations, including solid dose forms, capsules, gummies, soft chews, powders, and liquids. A 2019 consumer survey by the Council for Responsible Nutrition (CRN) revealed that dietary supplement usage in the US population was at its all-time highest: 77% of Americans reported consumption of dietary supplements [2]. A majority of adults 18 years and above (both male and female) consume dietary supplements and the highest usage of dietary supplements was reported between ages 35 and 54 at 81 percent.

A report by the US Accountability Office documented that the number of dietary supplements in the market had grown from around 4,000 products in 1994 to 80,000 products in 2016: an exponential growth with the addition of nearly 3500 products every year. Concomitant with this increase, a new set of supplements have emerged - nootropics - those claiming to support brain health and address memory or memory loss. While these supplements comprise at this point a small segment of the market, their sales almost doubled from 2006 to 2015 ($353 million to $643 million). A report by the Global Council on Brain Health has forecast the global brain health supplement market size to grow by 2023 to nearly $5.8 Billion [3]. A 2019 survey (N=2,292 subjects) on Brain Health Supplements conducted by the American Association of Retired Persons (AARP) and Ipsos (New York, NY) showed that 23% of adults (18+ years old) consume at least one supplement for brain health and this figure increases to 36% in adults 74 years and older [4]. Nearly 23% of adults in the US (58 million) take supplements for maintaining or improving brain health or mental sharpness while around 8% (9 million) take supplements with the belief that supplements can reverse or delay dementia. A large majority of adults also believe that supplements are effective for maintaining and improving brain health, while 50% feel that supplements are somewhat effective in reversing dementia [4]. With the aging population on the rise and retaining mental sharpness with age being a health concern, it should not be a surprise then that adults are turning to dietary supplements for brain health and even hoping to prevent or treat age-related memory loss (a claim that dietary supplements are not allowed to make) [5].

While the AARP-Ipsos survey focused on brain health and mental sharpness, the other emerging area where dietary

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supplements could have an impact is the emerging area of nutritional psychiatry [6, 7]. The structure and function of the brain is critically dependent on nutrient availability such as lipids, amino acids, vitamins, and minerals. It logically follows, therefore that dietary intake and quality can be a strong lever to influence mood as well [8]. For example, individuals with low 25-hydroxy vitamin D levels have been found to suffer from a higher incidence of mood disorders [9]. Indeed, CRN's 2019 Consumer Survey on Dietary Supplements reported that 34% of consumers taking brain supplements do so for supporting their mood [2].

Thus, nootropic dietary supplements are appealing to consumers for cognition, focus, memory, and mood. Memory might be a longer-term benefit, whereas focus, concentration, alertness are shorter term noticeable benefits. While Fish Oil, and Omega-3, are the top brain health supplements, more than 10 million adults take proprietary/specialty brain supplements such as BriteSmart™, Cognifen, Cognitex®, and Prevagen® [4]. Within this subset, herbal and botanical ingredients are seeing rapid growth. Seventy-two percent of dietary supplement users are confident in the safety and quality of herbals and botanicals. Fifty percent of total supplement users include an herbal/botanical in their supplement regimen and this category has grown by 19% since 2015 [2]. Accordingly, in this review article, we discuss both safety and efficacy data for various herbal and botanical ingredients from a brain health perspective and focusing on both short- and long-term benefits.

2. ASSESSMENTS FOR COGNITIVE ENDPOINTS

As outlined in the introduction, nootropic dietary supplements are being positioned in the market and used by consumers to experience benefits in the areas of cognition,

focus, memory, concentration, alertness, stress, and mood. In this section and Table **1**, we review some of the standard assessments that are used to assess the efficacy of the supplements in these benefit spaces. In this context, for ease of a framework, cognition and memory fall into one bucket with focus, concentration and alertness as sub-components, and stress and mood fall into another bucket. Additionally, we have included data related to sleep in those few clinical studies in which sleep was also an endpoint of investigation.

2.1. Cognition and Memory

Cognition and memory are assessed using a neuropsychological battery of tests that determine cognitive strengths and weaknesses. These assessments aim to probe different cognitive domains each thought to be governed by a distinct anatomical structure in the brain. However, most cognitive tasks rely on a combination of processes and distributed neural networks [10]. These are standardized tests that evaluate different kinds of memory (associative, free recall, visual, memory span, and meaningful), attention, visual perception, auditory perception, language behavior, number facility, reasoning, mental speed, idea production and executive functions.

2.1.1. Attention and Processing Speed

Attention and processing speed are fundamental to any cognitive task. Formal tests for attention and working memory include digit span forwards and backwards (Weschler Memory Scale-IV Digit Span) and tests of vigilance and sustained attention. In the digit span test, the test-taker is required to repeat numbers either in the same order or reverse order of that shown on the screen or presented by an examiner [11].

Table 1.   Summary of standard assessments used to assess the efficacy of treatments for cognitive and memory endpoints.

Cognition and Memory		
Refs.	**Cognitive Endpoints**	**Specific Tests**
[11, 13, 14]	Attention and processing speed	Weschler Memory Scale-IV Digit Span, Trail Making Test and Weschler Adult Intelligence Scale (WAIS-IV) Symbol Search Test
[12, 15]	Vigilance and sustained attention	Conners' Continuous Performance Test II, Rapid Visual Information Processing (RVIP) task
[16, 17]	Memory (encoding, retention, retrieval)	Auditory Verbal Learning Test (AVLT), California Verbal Learning Test (CVLT), Weschler Memory Scale-IV logical memory
[18]	Visual and spatial perception	Visual Object and Space Perception Battery, Birmingham Object Recognition Battery
[10, 20-22]	Executive Function (flexible thinking, emotional control, self-monitoring, prepotent inhibition, planning and prioritizing)	Oral Word Association Test, Delis-Kaplan Executive Function System test, Stroop test
Mood and Stress		
Refs.	**Cognitive Endpoints**	**Specific Tests**
[27-30]	Anxiety	Modified Hamilton Anxiety Scale (mHAM), Perceived Stress Scale (PSS), Geriatric Depression Scale, Hospital Anxiety and Depression Scale (HADS), Profile of Mood States (POMS), Bond-Lader Visual Analogue Scale (BL-VAS), and multi-tasking framework (MTF)

The Conners' Continuous Performance Test II is often used to measure vigilance and sustained attention. The participant presses the space bar or clicks the mouse button when a letter other than X shows up onscreen. Letters appear on the screen with different time intervals between each one. Exactly 14 minutes is required for completion [12].

Processing speed is often measured by timed tasks such as the Trail Making Test and Weschler Adult Intelligence Scale (WAIS-IV Symbol Search Test). In the Trail Making Test, the subject has to connect a set of 25 dots as quickly as possible while maintaining accuracy [13]. In the WAIS-IV symbol search test, each item is presented sequentially in a row. In each row, two symbols are presented to the left of a set of five symbols. The correct answer is yes if the set of five symbols includes either of the two symbols on the left. Matches occur at a rate of 50%, and total correct positive and negative responses are tallied for the raw score [14].

The Rapid Visual Information Processing (RVIP) task is a serial detection task used to study visual sustained attention (*i.e.*, vigilance) and working memory processes [15]. The RVIP measures the effect of a secondary signal-detection task on performance in a primary signal-detection task in an auditory vigilance scenario. As example, participants may listen to a recording of a sequence of digits in order to detect the occurrence of primary signals such as successive odd digits which are all different. In another scenario, participants listen to the primary signals as defined previously, but in addition, they must detect secondary signals defined as the occurrence of, for example, the digit 6. The overall detection of primary versus secondary signals, or both, can then be compared. Thus, these tests described in this section can be used to determine efficacy of a nootropic regarding attention, focus, concentration, accuracy and speed.

2.1.2. Memory

Memory tests typically evaluate episodic memory encompassing the ability to consolidate new information (encoding), the ability to retain this information over time (retention), and ability to recall information after a delay or interference (retrieval). Tests to assess episodic memory include word list learning tasks such as the Auditory Verbal Learning Test (AVLT), California Verbal Learning Test (CVLT), recall of stories (Weschler Memory Scale-IV logical memory), verbal paired associates (two words are paired and subject has to recall one word when the other is given as the cue, and recall of complex geometric figures [16]. In the AVLT and CVLT, five presentations of a 15-word list are given, each followed by attempted recall. This is followed by a second 15-word interference list (list B), followed by recall of list A. Delayed recall and recognition are also tested. These tests also determine the rate of learning since multiple stimuli are presented [17].

2.1.3. Visual and Spatial Perception

Visual and spatial perception assesses interpretation of simple and complex visual stimuli. Two commonly used tests are the Visual Object and Space Perception Battery and the Birmingham Object Recognition Battery. These tests contain tasks on orientation, size, length, position of objects,

recognition of objects (*e.g.*, incomplete letters, silhouettes) and spatial relations (*e.g.*, position discrimination) [18].

2.1.4. Language

Language component of cognition evaluates spontaneous speech, semantic knowledge, grammar, reading of regular and irregular words, and repetition of words and sentences [19].

2.1.5. Executive Function

Executive function is an overarching term referring to the higher-level cognitive skills such as flexible thinking, emotional control, self-monitoring, prepotent inhibition, planning and prioritizing that are used to control and co-ordinate other cognitive skills and behaviors [10]. Executive function is assessed using tests such as word generation tasks (*e.g.*, controlled Oral Word Association Test - producing as many words as can begin with a given letter within 1 minute [20], planning (Delis-Kaplan Executive Function System test - composed of nine tests including trail making test, verbal fluency, proverb test, *etc.*) [21], inhibition (Stroop test - saying the color of the word and not what the word is) [22].

2.1.6. Abbreviated Cognitive Screening Tools

While the above tests are comprehensive assessments, they are also time-consuming. To overcome this challenge, other abbreviated cognitive screening tools are also used such as the Cambridge Neuropsychological Test Automated Battery [23]. This is a set of 25 tests that measure memory, learning, working memory, executive function, attention and reaction time, decision making, response control, semantic/verbal memory and can be administered using a touchscreen computer. Another computer based automated assessment that is commonly used is the Cognitive Drug Research (CDR) battery [24]. Tests available in this system include attention (simple reaction time, choice reaction time, digit vigilance), executive function and working memory (semantic reasoning, rapid visual information processing), episodic secondary memory (word recall, word recognition, picture recognition), and motor control (joystick tracking task, tapping task). The primary advantage of the Cambridge Neuropsychological Test Automated Battery and CDR battery tests lies in their ability to automate the tests either *via* a central computer or the internet automatically and simultaneously testing hundreds of patients in any location without the involvement or need for additional study personnel and paperwork thereby greatly facilitating remote assessment of cognitive function. Other tests include the Mini-Mental State Exam (MMSE) [25] - a 30 point test used to measure thinking ability (repeat words, simple mathematical problems, recall names of 3 objects learned earlier, obey written instruction) and the Montreal Cognitive Assessment - again a 30 point test that is newer than the MMSE and includes a clock-drawing test (*e.g.*, person is asked to draw a clock that reads ten past eleven) and is not as heavily weighted on language as the MMSE [26].

2.2. Mood and Stress

Mood and stress are measured using subjective self-reports scaled to measure levels of anxiety. Common scales

include the modified Hamilton Anxiety Scale (mHAM) (also referred to as the modified Hamilton Depression Rating Scale) [27], Perceived Stress Scale (PSS) [28], and Geriatric Depression Scale, Hospital Anxiety and Depression scale (HADS) [29], Profile of Mood States (POMS), Bond-Lader Visual Analogue Scale (BL-VAS) [30], and multi-tasking framework (MTF).

2.2.1. Hamilton Anxiety Scale (mHAM)

The mHAM questionnaire is a self-rated 5-point scale (0 = no symptoms; 1 = occasional; 2 = mild/poor; 3 = moderate; 4 = severe) of the following symptoms of stress/anxiety: fatigue, flushing, perspiration, loss of appetite, headache and muscle pain, feelings of impending doom, palpitations, dry mouth, sleeplessness, forgetfulness, irritability and inability to concentrate.

2.2.2. Perceived Stress Scale (PSS)

The PSS measures the degree to which situations are appraised as stressful by the participant. This includes 10 questions such as "In the last month, how often have you been upset because of something that happened unexpectedly?". Answers go from 0 to 4 (Never to Very Often).

2.2.3. Geriatric Depression Scale

The Geriatric Depression Scale consists of questions that assess a participant's level of enjoyment, interest, social interactions. Examples of questions on the scale include "Are you basically satisfied with your life, have you dropped many of your activities and interests, do you feel that your situation is hopeless?". Each question has yes/no answers.

2.2.4. Hospital Anxiety and Depression Scale (HADS)

The HADS questionnaire comprises seven questions for anxiety (*e.g.*, "I feel tense or wound up") and seven questions for depression (*e.g.*, "I can enjoy a good book or radio or TV program") and takes 2-5 minutes to complete. Anxiety and depression are scored separately.

2.2.5. Profile Mood States (POMS)

The POMS is a rating scale and measures distinct mood states [31]. There are two variants: long (65 words or statements that describe feelings) and short form (35/37 words or statements). This test can take 5-15 minutes to complete. Examples of feelings include friendly, tense, angry, unhappy, lively, confused and the participant can choose from not at all, a little, moderately, quite a bit, extremely to rate each feeling.

2.2.6. Multi-tasking Framework (MTF)

The MTF is a performance-based assessment of how individuals respond to several different stimuli with varying levels of workload [32]. There are eight generic cognitive tasks. Acute stress is created either by increasing the number of tasks or the individual difficulty of those tasks for example, calculating values, the continuous monitoring of displays and auditory information inputs and the need to react to specific stimuli whilst ignoring less relevant information. Psychological (mood and perceived workload) and physiological (heart rate and blood pressure) are observed as participants are exposed to a 15-minute period of multitasking

at different workload levels. Since multitasking is typical in a real-life setting, this framework provides a technique to measure acute stress and workload in the laboratory.

2.2.7. Bond-Lader Visual Analogue Mood Scale (B-L-VAS)

The B-L VAS is a method to self-evaluate mood. Fifteen dimensions of mood are measured, such as Alert-Drowsy, Calm-Excited, Strong-Feeble, Happy-Sad. The participants mark on a 100 mm line to what extent they relate to the described state at that moment. The responses from 16 mood scales are combined to make dimensions of alertness, contentment, and calmness [30].

3. BOTANICAL NOOTROPICS

The botanical ingredients discussed herein were chosen using a market assessment conducted to identify nootropic products sold by major retailers. From this assessment, we chose to focus our review on *Bacopa monnieri*, *Ginkgo biloba*, *Ocimum tenuiflorum* (Holy basil), *Panax quinquefolius* (American ginseng), *Centella asiatica* (Gotu kola), *Melissa officinalis* (Lemon balm), *Salvia officinalis*, *Salvia lavandulaefolia* (Common and Spanish sages), and *Mentha spicata* (spearmint).

3.1. Search Strategy

The following databases were searched for relevant publications: PubMed, Web of Science and Google Scholar, EMBASE (which also searches PubMed and Medline) and Biosis. There were no publication date limits placed on the searches of these databases. Database searches were conducted separately for each of the botanicals of interest. To obtain articles relevant to clinical efficacy, the article type 'clinical efficacy' was used with search terms 'Genus species (*e.g.*, *Melissa officinalis*)' or 'common name (*e.g.*, lemon balm)' AND 'focus' OR 'concentration' OR 'attention' OR 'mood' OR 'stress' OR 'stress reduction' OR 'memory'. To obtain articles relevant to clinical and non-clinical safety, the article type 'clinical safety' OR 'non-clinical safety' was used with search terms 'Genus species (*e.g.*, *Melissa officinalis*)' or 'common name (*e.g,*. lemon balm)' AND 'tolerable' OR 'toxicity' OR 'toxicology' OR 'acute' OR 'mortality' OR 'lethal dose' OR 'sub-chronic' OR 'chronic' OR 'NOAEL' or 'organ toxicity' OR 'developmental' OR 'reproduct*' OR 'teratogenic' OR 'teratology' OR 'fertility' OR 'embryo' OR 'fetal' OR 'malformations' OR 'perinatal' OR 'postnatal' OR 'abortifacient' OR 'genotox*' OR 'genetic' OR 'mutagen*' OR 'mutation' OR 'carcinogen' OR 'tumor' OR 'bioassay'. All searches were conducted in March and April of 2020.

3.2. Selection of Studies

Once literature searches of the various databases were complete, abstracts were manually reviewed and filtered by the authors to select those studies that focused on the effect of a single botanical on the cognition parameters of interest. Similarly, for non-clinical data, only toxicology studies on single botanicals were used. Data were extracted from the literature into Microsoft Excel spreadsheets. The following study parameters for clinical studies were extracted from the abstracts, and when necessary the full publications: study

design, outcome(s), outcome tool(s) used, subject demographics, dosing, botanical characterization information, results, adverse events, and Jadad score. The following study parameters for non-clinical studies were extracted from the abstracts, and when necessary, the full publications: study type, toxicity endpoint(s), study details, dosing, botanical characterization information, and study results.

Clinical studies were assessed for quality using the method described by Jadad *et al.* [33]. A J-score was provided for each study reviewed (J=1-5). Only those studies with a J-score of 4 or 5 are discussed within this review.

4. EFFICACY AND SAFETY OF BOTANICAL NOOTROPICS

4.1. *Bacopa monnieri*

Bacopa monnieri L. (family Scrophulariaceae) is a small creeping succulent that has been used for centuries in Ayurvedic medicine to improve memory and intellect [34]. The principal phytochemical constituents hypothesized to be associated with the pharmacological action of *B. monnieri* are bacoside-A and bacoside-B.

There are two commercialized extracts of *B. monnieri* that have been the most well-studied in clinical trials for beneficial effects on memory and cognition: KeenMind® and BacoMind™. KeenMind® contains 150 mg of an ethanolic extract produced from the aerial parts of *B. monnieri* and is known as CDRI 08. The CDRI 08 extract is standardized to not less than 55% bacosides. BacoMind™ is an ethanolic extract standardized to at least 45% bacosides [35].

4.1.1. *Bacopa monnieri* Clinical Efficacy

In a randomized, double-blind, placebo-controlled study, 46 healthy subjects between the ages of 18 and 60 years were given one KeenMind® capsule (150 mg CDRI 08 extract) or placebo twice a day for 12 weeks to observe cognitive function [36]. The outcome measures used were digit symbol substitution, speed comprehension, digit span, trial making, AVLT, and Inspection Time (IT). The authors concluded that KeenMind® improved speed of visual information processing, learning rate, and memory consolidation measured by AVLT and IT (p<0.05) at 12 weeks. These findings suggested that the botanical extract may improve higher cognitive processes. The authors observed that the types of adverse effects were similar between the treatment and placebo groups, as summarized in Table **2**. The only adverse effects that were higher in the KeenMind® treatment group were dry mouth, nausea, and muscular fatigue.

A second clinical study was conducted by these same sponsors in a double-blind, placebo-controlled study with 107 healthy subjects aged 18-60 [37]. Study subjects were randomized to receive 150 mg KeenMind® extract capsule or placebo twice a day for 90 days to observe treatment on cognitive function. The outcome measures in this study included CDR and a rapid visual information-processing (RVIP) task. Treatment with KeenMind® improved working memory (p=0.035) and RVIP improved (P=0.029) at 90 days. No other CDR measures for secondary memory, speed of memory, speed attention and accuracy of attention reached significance. The study sponsors concluded that

KeenMind® improved working memory performance, specifically spatial working memory in healthy adults after 90 days of treatment. Additionally, visual perception through RVIP improved specifically through performance accuracy. Authors suggested both treatments were well tolerated. The KeenMind® treated group had 22 dropouts and placebo group had 23 dropouts, with 3 dropouts in the KeenMind® group and 2 in the placebo due to adverse events. The authors concluded that KeenMind® treatment improved cognitive function.

In a randomized, double-blind, placebo-controlled study, 24 healthy subjects between the ages of 18-56 years were administered 4 capsules of placebo, 320 mg, or 640 mg KeenMind® extract capsules to study acute cognitive effects of *B. monnieri* [38]. The cognitive endpoints were measured using a Cognitive Demand Battery (CDB) which included a serial subtraction of 3s and 7s, and RVIP. Subjects were required to attend four sessions, one practice and three study visits, once a week to ensure washout between each dose. During each visit, one of the treatments was administered and the volunteers completed a CDB test. Significantly better performance scores over baseline were measured with treatment of 320 mg KeenMind® during the first, second, and fourth repetition post-dosing on the CDB tasks (p<0.05). Treatment had no effect on cardiovascular activity (blood pressure) which was used as an indicator of stress. There were no significant changes in the RVIP assessment. Interestingly, there was no significant benefit in study endpoint measures from the higher dose of 640 mg KeenMind®. There were no adverse events reported in the study.

Benson *et al.* conducted a small-based randomized, double-blind, placebo-controlled study in 17 healthy subjects ages 18-44 years old [39]. A Multi-tasking Framework (MTF) which included mental arithmetic, Stroop, letter search, and visual tracking were the efficacy endpoints studies. Treatments given included placebo, 320 mg or 640 mg KeenMind® per day; and subjects were evaluated at weeks 0, 1, 2, and 3 at 2 hours post-dose. Treatment with KeenMind® at both 320 mg and 640 mg resulted in higher scores for both Stroop and letter search at 1- and 2-hours post-treatment. There were no significant treatment-related changes in mental arithmetic and visual tracking. The authors concluded that given the results for the Stroop task and letter search performance, KeenMind® may improve faster information processing and decision making.

In a randomized, double-blind, placebo-controlled study, 98 healthy subjects over the age of 55 were administered 300 mg/day BacoMind™ or placebo once a day after meals for 12 weeks to observe memory performance improvement in healthy older persons [40]. The authors concluded that BacoMind™ treatment significantly improved memory retention and acquisition (p=0.001 for delayed recall; p=0.011 for total learning; and p=0.048 for retroactive interference). 13 volunteers in the BacoMind™ group dropped out; 9 were due to side effects including, increased stool frequency, gastrointestinal cramps, and nausea.

In a randomized, double-blind, placebo-controlled study, 65 subjects between 50-75 years of age with complaints of memory impairment for at least one year were randomized to

Table 2.   **Adverse effects recorded in a 12-week clinical study in which subjects were treated with either *Bacopa monnieri* or placebo, and as reported by Stough *et al*. [36].**

Adverse Effect	Bacopa Monnieri (300 mg/day)	Placebo
Drowsiness	5%	4%
Allergies	14%	16%
Cold/flu symptoms	9%	28%
Skin rash	5%	12%
Skin itching	5%	12%
Headache	18%	28%
Tinnitus	9%	16%
Vertigo	9%	8%
Strange taste in mouth	14%	16%
Dry mouth	23%	16%
Palpitations	18%	8%
Abdominal pain	9%	8%
Appetite increase	18%	20%
Appetite reduction	0%	4%
Excessive thirst	18%	8%
Nausea	18%	4%
Indigestion	9%	4%
Constipation	9%	8%
Increased regularity of bowel movements	9%	4%
Increased frequency of urine	14%	8%
Muscular fatigue	14%	4%
Muscular pain	5%	8%
Cramps	5%	8%
Increase in felt stress	9%	12%
Decrease in felt stress	23%	28%
Improved mood	5%	8%
Worsened mood	5%	8%

receive 450 mg BacoMind™ tablet or placebo once daily for 12 weeks, followed by an additional 12-week washout period to observe memory improvement maintenance [41]. Tablets of BacoMind™ were taken once daily after breakfast with water. The study sponsor reported no serious adverse events throughout the study. No mild or moderate adverse events were reported or explored by the investigator. The authors reported three individuals dropped out of the study, two in the placebo and one in the treatment. None of the dropouts were due to adverse events. Authors concluded that treatment with BacoMind™ improved cognitive functions in the study participants.

4.1.1.1. Other Standardized *Bacopa monnieri* Extracts

Robust clinical studies have been conducted with other standardized extracts of *B. monnieri*. Calabrese *et al.* conducted a randomized, double-blind, placebo-controlled trial in 54 health subjects over the age of 65 [42]. The primary cognitive function endpoint studied was AVLT, and secondary endpoints of Stroop Task and WAIS. Subjects were given 300 mg of a standardized *B. monnieri* extract for 12 weeks; and evaluated at 0, 6, and 12 weeks, and again at 18 weeks (post-treatment period). The proprietary *B. monnieri* used in this study was extracted with methanol: water (70:30) to produce a 50:1 dry extract with a minimum of

50% bacosides A and B. Both the AVLT and Stroop Task reaction times improved (p<0.05) after 12 week of dosing. There was no significant change found in WAIS. The study sponsors concluded that *B. monnieri* extract can enhance cognitive function in aging populations after 12 weeks of treatment. A total of 41 adverse events were reported in the study, 23 in placebo and 18 in the *B. monnieri* group. Symptoms primarily included flu-like and gastrointestinal issues.

Another standardized extract of *B. monnieri* (Bacognize®) was used in a randomized, double-blind, placebo-controlled, non-crossover, parallel design trial to study cognitive functions including immediate recall, recognition, working memory, attention, associative abilities, reasoning, transformation, and language comprehension [43]. These endpoints were studied using various neuropsychological tasks including a digit span memory task, logical memory test, memory span for nonsense syllable, finger tapping test, simple and choice reaction time, choice discrimination test, and digit picture substitution test. A total of 60 medical students (19-22 years) were given placebo or 150 mg of Bacognize® (standardized to 45% bacosides) twice daily for 6 weeks and evaluated at weeks 0 and 6 with a 15-day follow-up. Two out of the 10 neuropsychological tasks assessed by digit span backward and logical memory had significant improvement (p<0.05) after 6 weeks of treatment with *B. monnieri*. The authors concluded that *B. monnieri* may be beneficial to some components of memory with only 6 weeks of administration.

4.1.2. Bacopa monnieri Non-clinical Safety

There are limited non-clinical animal studies that have been conducted with various extracts of *B. monnieri*. A sub-chronic 90-day study in rats tested with BacoMind™ at doses up to 500 mg/kg bw/day showed no changes in body weight, clinical signs, hematological and blood chemistry, histopathology, and food consumption [44]. A NOAEL of 500 mg/kg bw was identified from this study.

A chronic (270-day) study in rats showed no toxicity in hematological and biochemical parameters, body weight, and gross pathology up to 1500 mg/kg bw/day of *B. monnieri*; the highest dose tested [45].

A reproductive toxicity study in male mice fed with the whole *B. monnieri* plant extract doses up to 80 mg/kg for 28 days showed no difference in body weight, testis, epididymis, and seminal vesicle weights but increases in sperm viability and spermatogenic cell density [46].

The standardized BacoMind™ extract has been well studied in genotoxicity assays for both mutagenicity and clastogenicity [47]. No potential for mutagenicity was observed in an Ames assay. Negative results were also obtained in an *in vitro* chromosomal aberration assay, and an *in vitro* micronucleus assay; both using human blood lymphocytes. All three studies to assess genotoxicity were conducted according to the Organisation for Economic Co-operation and Development (OECD) guidelines.

4.2. Ginkgo Biloba

Ginkgo biloba L., (Ginkgoaceae Engl.) leaf extract is one of the most popular herbal supplements that is used to im-

prove cognition, mood, attention, as well as enable positive effects on circulation. In 2008 it was reported that sales *of G. biloba* extract products were as high as $249 million in the US alone [48]. The phytochemical constituents considered active in *G. biloba* extracts include flavonol glycosides, terpene lactones, and ginkgolic acids. One of the most common and well-studied standardized extracts of *G. biloba* is EGb 761®, which contains 24% flavonoids and 6% terpenes ginkgolides and bilobalide. Common doses of EGb 761® often used in clinical studies are 120 mg per day or 240 mg per day (120 mg twice daily). A summary of clinical efficacy trials using EGb 761® to study cognitive function which included memory, learning, mood, anxiety and attention outcomes, can be found in Table **1**. Select studies in which EGb 761® has had some positive effects on cognitive function in adults suffering from dementia or Alzheimer's, older adults without neurocognitive deficits, and young health adults are summarized below. Multiple other studies with EGb 761® failed to demonstrate enhancement of cognitive functions whether in single-dose studies or long-term studies (Table **3**).

4.2.1. Ginkgo Biloba Clinical Efficacy

G. biloba has long been studied in dementia and Alzheimer's patients. One of the largest studies was conducted by DeKosky *et al.*, which recruited 3,072 eligible individuals with normal cognition or mild cognitive impairment (MCI) from 2000 to 2008 [48]. Baseline MCI was defined by having at least 2 of 10 selected neuropsychological test scores from each cognitive domain, including memory, language, visuospatial abilities, attention, and executive function; and Clinical Dementia Rating global score of 0.5. Participants in this large randomized, double-blind, placebo-controlled trial included those with normal cognition (n=2587) and MCI (n=482) randomized in a 1:1 ratio for twice-daily doses of 120 mg of EGb 761® (n=1545) vs. placebo (n=1524). Results showed no significant difference in the rate of dementia between *G. biloba* group and placebo. There was also no difference between EGb 761® treated and placebo group in the rate of Alzheimer's disease during this period. In conclusion, this large sample-size, long-term study showed that *G. biloba* was ineffective in reducing the overall incidence rate of dementia or Alzheimer's disease.

Kaschel, R. *et al.*, assessed similar cognitive endpoints with healthy middle-age (45-56-year-old) individuals to compare EGb 761® extract (240mg/day) effects in memory performance versus placebo for 6 weeks [49]. A randomized, double-blind, placebo-controlled trial was performed in a 1:1 ratio to determine whether subjects show improvement in two memory tests, free recall (list of appointments) test and recognition (driving-route) test. The findings indicated that EGb 761® treated group had improved quantity of free recall, *i.e.*, the number of correctly recalled appointments significantly compared to placebo (p = 0.038 for immediate and p = 0.008 for delayed recall). No significant difference was shown in a less demanding everyday memory (recognition) task. The authors concluded that EGb 761® showed specific patterns of benefit in terms of improving a higher level of cognitive functioning.

Clinical trials to determine whether *G. biloba* could improve cognitive function in healthy individuals have also

Table 3.　　**Summary of efficacy clinical studies with the standardized *Ginkgo biloba* extract EGb 761®.**

Refs.	Study Design	Endpoints	Dosage & Duration	Results
[48]	RDBPC[a]	Prevention of dementia	240 mg/day (120 mg BID) *vs.* placebo x 2 years	No significant difference.
[49]	RDBPC	Cognitive function - memory tests	240 mg/day *vs.* placebo x 6 weeks	No significant difference.
[50]	RDBPC	Mood	40 mg or 80 mg per day film-coated tablets *vs.* placebo x 4 weeks	Significant superiority of EGb 761®over placebo on all secondary outcome measures.
[51]	RDBPC, parallel	Cognitive function	120 mg/day (40mg TID) *vs.* placebo x 52 weeks	EGb 761® stabilized and/or improved cognitive performance and social functioning of dementia patients for 6 months to 1 year.
[52]	RDBPC, mono-center	Cognitive function, Mood	240 mg TID *vs.* placebo x 4 weeks.	Intergroup differences in self-estimated mental health and self-estimated quality of life were significant with EGb 761®.
[53]	RDBPC	Cognitive function, Memory	120 mg/day *vs* placebo x 30 days	EGb 761® improved memory processes, particularly working memory and memory consolidation.
[54]	RDBPC, fixed-dose	Cognitive function	180 mg/day *vs.* placebo x 6 weeks	EGb 761® did not show significant changes in objective measures of memory. There were significant improvements in Stroop color and word tests.
[55]	RDBPC	Cognitive function	240 mg/day *vs.* placebo x 12 weeks	No significant difference.
[56]	RDBPC	Cognitive function	120 mg/day *vs.* placebo x 1 dose	No significant difference.
[57]	RDBPC, cross-over	Cognitive function	120 mg/day (40 mg three times daily) *vs.* placebo daily x 12 weeks	Differential improvement in performance with EGb 761 on delayed recall for long-term storage and retrieval measure and inspection time. No differences between the two groups for POMS. Possible beneficial cognitive enhancement in EGb 761 group for older population.
[58]	RDBPC, parallel	Cognitive function	120 mg/day (40 mg/mL) *vs.* placebo x 1 dose	No significant difference.
[59]	Open-label, observational study	Mood	200 mg/day x 4 weeks	Preliminary result shows potential benefit to use Ginkgo biloba for ADD treatment.
[60]	RDBPC, multi-center	Cognitive function	240 mg/day *vs.* 160 mg/day *vs.* placebo x 24 weeks	No significant difference.

[a]Randomized, double-blinded, placebo-controlled.

been studied. Mix *et al*. studied the effects of EGb 761® in older individuals without a history of neurocognitive dysfunction [54]. Forty-eight subjects between the ages of 55 and 86 years participated in a 6-week study where they were administered 180 mg per day of EGb 761® in a randomized placebo-controlled, double-blind study. The cognitive function outcomes included assessing speed of processing abilities (*i.e.*, Stroop Color and Word Test color-naming task). Results for treatment with EGb 761® were significant for the Stroop Color and Word Test, and trending towards favorable results in other tasks assessing a timed speed of processing component. No significant differences were found between the treatment and placebo groups on objective memory measures assessed using the Wechsler Memory Scale-Revised.

The neurocognitive enhancing ability of *G. biloba* has also been studied in younger adults. Stough *et al*., studied whether EGb 761® could improve various neuropsychological functioning in 61 young healthy adults (18-40 years) [53]. Subjects in this randomized, double-blind, placebo-controlled clinical trial were given 120 mg per day EGb 761® for 30 days. A battery of well-validated tests was used to assess attention, working and short-term memory, verbal learning, memory consolidation, executive processes, planning and problem solving, information processing speed, motor responsiveness, and decision making, after a chronic regimen of EGb 761®. At the conclusion of the study, EGb 761® significantly improved speed of information processing working memory and executive processing.

Fewer studies have been conducted to determine whether EGb 761® can have a positive effect on mood. Woelk, H., *et al*., conducted a randomized, double-blind, placebo-controlled study enrolled 107 participants with a generalized anxiety disorder (N=82) or adjustment disorder with anxious mood (N=25) to find out if *G. biloba* had potential in improving mood and alertness [50]. Subjects were randomized to a daily dose of either EGb 761® 480mg/day, EGb 761® 240mg/day, or placebo for 4 weeks. The mHAM, the clinical global impression of change (CGI-C), the Erlangen anxiety tension and aggression scale, the list of complaints, and the patient's global rating of change were utilized. Results showed mHAM total scores decreased by -14.3 (± 8.1, p=0.0003) and -12.1 (± 9.0, p=0.01) in high-dose EGb 761® and low-dose EGb 761®; respectively compared to placebo group -7.8 (± 9.2). EGb 761® demonstrating potential long-term effects in the improvement of mood and alertness.

As mentioned previously, *G. biloba* extracts have been widely used globally for decades. It has been estimated that approximately 1 million doses of EGb 761® are sold per day [61]. As a result of this significant human exposure to *G. biloba*, it is critical that safety and tolerability is thoroughly assessed. Heinonen and Gaus recently utilized a cross matching tool that combined toxicological and clinical data, along with other sources of information for *G. biloba* to assess human safety [61]. Utilizing 75 high quality clinical trials that included 7115 patients treated with *G. biloba* and toxicological data (*e.g.*, the Technical Report TR 578 of the US National Toxicology Program), these authors determined that *G. biloba* is well tolerated and safe for humans. Looking at potential adverse events reported across the 75 studies, the authors concluded that there were no specific or serious reports noted for G. biloba, and any adverse events reported had a similar frequency as placebo. This is consistent with the studies we reviewed herein for EGb 761®.

4.2.2. Ginkgo biloba Non-clinical Safety

The US National Toxicology Program (NTP) tested a *G. biloba* leaf extract in a 105-week study in rats and mice (NTP Technical Report) [62]. At the conclusion of the study, there was an increase in liver tumors in male and female mice and in thyroid tumors in male and female rats and male mice. As described in the NTP Technical Report, genotoxicity studies with *G. biloba* extracts have resulted in equivocal results. Earlier three-month toxicity studies performed in rats and mice by this same group indicated that the liver and thyroid were target organs of toxicity [62].

In a later study to determine how the doses used in the NTP bioassay were to human doses from use of *G. biloba* dietary supplements, Waidyanatha *et al*., evaluated the systemic exposure in rats after single doses of *G. biloba* extracts (30, 100, and 300 mg/kg) [63]. Systemic exposure in rats of terpene trilactones were monitored, and dose-normalized maximum plasma concentration (Cmax) and area under the curve (AUC) values were determined to be within 5-fold of published rodent studies. Similarities of rat systemic exposure data at a dose of 100 mg/kg/day to published human data following ingestion of 240 mg *G. biloba* suggest the relevance of NTP rodent toxicity data to humans.

Since the NTP bioassay results for *G. biloba*, other authors have conducted studies to further assess the genotoxicity potential of extracts. Maeda *et al*. have performed reporter gene mutation assays using *gpt* delta mice; and a combined liver comet assay and bone marrow micronucleus assay using C3H-derived constitutive androstane receptor knockout (CARKO) and wild-type mice [64]. *In vivo* doses in both studies were up to 2000 mg/kg bw/day. Both studies indicated no genotoxicity, and the authors conclude that *G. Biloba*-induced hepatocarcinogenesis in mice, as reported in the NTP studies, occurs through a non-genotoxic mode of action. Notably, Maeda *et al*., used the same *G. biloba* extract (and lot) as was used in the NTP bioassay [64].

These same authors published another study a year later after assessing potential hepatocarcinogenesis associated with *G. biloba* in CARKO and wild-type mice [65]. The study conducted an evaluation at 1-week, 4 or 13-week, and 27-week of the treatment period. There was an increase in hepatocellular DNA replication in WT mice at 1-week. Hepatocellular hypertrophy and induction of CYP2B10 were observed in WT mice in 4 or 13-week compared to a smaller effect in CARKO mice. An increase of eosinophilic altered foci and adenomas were observed in 27-week treatment with GBE group WT mice. No significant increase in foci and adenomas were showed in CARKO mice. Findings indicate that GBE-induced hepatocarcinogenesis appears highly CAR-mediated.

Koch *et al*., investigated the reproductive and developmental toxicity of the EGb 761® using 100 female CD-1 mice with a dose of 100, 350, and 1225 mg/kg bw/day [66]. Mice were sacrificed at day 17 of gestation to examine the potential toxicity on embryo-fetal development during the critical period of organogenesis. No embryotoxicity was observed during external and internal inspection of the fetuses, skeletal, and soft tissues. No significant incidence of malformations was observed. The general condition of dams was not influenced, and the no-observed effect level was above 1225 mg/kg bw/day for both the dams and the fetuses. Necropsy revealed no pathological findings in the dams. No abnormality was observed in the external appearance of the dams after GBE dose with 100, 350, or 1225 mg/kg bw/day. Thus, EGb 761® showed no toxicity toward the developing fetus in mice.

4.3. Ocimum tenuiflorum (Holy basil)

Ocimum tenuiflorum L., commonly known as Holy basil, is an herb or shrub indigenous to India and parts of north and eastern Africa, Hainan Island, and China (Taiwan). It should be noted that *O. tenuiflorum* L. is the correct and preferred name; however, many publications still use the synonym *O. sanctum* [67]. Holy basil is a sacred plant in India where it has been used to treat numerous symptoms including cough, upper respiratory infections, skin infections, arthritis, asthma, common cold, diabetes, peptic ulcer, heart disease, malaria, and general stress [67, 68].

4.3.1. Ocimum tenuiflorum Clinical Efficacy

There are only two well-conducted clinical trials with Holy basil that study efficacy endpoints related to cognitive

function. Both studies were randomized, double-blind, and placebo controlled. In the first, conducted by Saxena *et al.*, 158 subjects who were suffering from three or more symptoms of stress were treated with a standard extract of whole plant *O. tenuiflorum* Linn. [69]. The standardized extract known as OciBest® (Natural Remedies Pvt. Ltd., Bangalore, India), complied with phytochemical specifications including ociglycosdie-I (hydroxychavicol glucoside/4-allyl-1-O-β-D-glucopyronosyl-2-hydroxybenzene; >0.01% w/w), rosmarinic acid (>0.2% w/w), and triterpene acids (>2.5% w/w). Each OciBest® capsule contained 400 mg of standardized phytochemical constituents, and subjects were treated with 1200 mg/day (3 capsules) for six weeks. The overall improvement on symptom scores of stress in the OciBest® group was 1.6 times or 39% greater than placebo. Treatment with the Holy basil extract was considered well-tolerated and there were no adverse events reported during the study.

Sampath and colleagues studied the effect on cognitive function of 300 mg/day capsules of an ethanolic leaf extract of Holy basil given to healthy male subjects (N=44) for 30 days [70]. The Holy basil extracts were prepared as a 70% ethanolic extraction from dried leaves (termed *Ocimum sanctum*, in this manuscript), and standardized to ursolic acid >2.7% w/w. The cognitive function tests administered included Sternberg and Stroop task, and event related potential. A comparison between the treatment group and placebo revealed a significant improvement in cognitive parameters with Holy basil treatment including reaction time and error rate of Sternberg test, and reaction time of neutral task of Stroop, reaction time and error rate of interference task of Stroop.

Additionally, the study by Sampath *et al.* measured markers related to stress including P300 latency (electroencephalogram measurement), salivary cortisol level, and State-Trait Anxiety Inventory [70]. There was an improvement over time in these parameters for subjects treated with Holy basil. There were no changes in the additional parameters of heart rate or galvanic skin response between treatment and placebo group. The authors concluded that Holy basil leaf extract seemed to have potential cognition-enhancing properties in humans. No adverse events were reported from this study. The authors did mention that previous experience with 300 mg doses of Holy basil was well tolerated and without adverse events.

Long-term oral exposure of *O. tenuiflorum* leaf extracts can be supported by a significant history of use in the diet, including food (*e.g.*, Thai cuisine) and in tea preparations. It also has a long history of use in Ayurvedic medicine and Traditional Chinese Medicine. Due to a lack of data for pediatric use, traditional use as an abortifacient, and conflicting reports on embryotoxicity of *O. tenuiflorum* L., it is not recommended for use by children or women who are pregnant and/or lactating, at doses exceeding expected daily intake *via* the diet [68, 71].

From a clinical safety perspective, *O. tenuiflorum* extracts appear to be quite safe; even at levels up to 1200 mg/day as reported by Saxena *et al*. In numerous well-conducted clinical studies for a variety of efficacy endpoints there are rare adverse events reports, and when reported only mild symptoms of nausea [68, 72].

4.3.2. Ocimum tennuiflorum Non-clinical Safety

In an OECD-compliant 90-day oral toxicity study in Wistar rats, lyophilized aqueous extracts of *O. tennuiflorum* L. was dosed at 250, 500, and 1000 mg/kg bw/day [73]. There were no significant toxicities or mortality observed in the study, and body weight gain, food consumption, hematology, biochemistry, organ weights, and histopathological examination of organs and tissues were considered normal across treatment groups.

As mentioned previously, Holy basil extracts are not recommended for use during pregnancy and/or lactation. *Ocimum tenuiflorum*, dry leaf extract is included in Annex 3 of the European Food Safety Authority (EFSA) advice on the draft guidance document on the safety assessment of botanicals and botanical preparations with possible safety issue of reproductive toxicity highlighted [67]. It is clear that reproductive and developmental toxicity due to use of *O. tenuiflorum* is a matter of concern for EFSA. As mentioned in the opinion by EFSA, the studies on reproduction and fertility are inadequate. Thus, there is a data gap for this toxicity endpoint, and clarification is needed on the teratogenic and reproductive potential for Holy basil extracts. The Health Canada monograph for *O. tenuiflorum* lists a contraindication for use if pregnant [74].

Extracts of *O. tenuiflorum* leaves have been studied for mutagenicity and clastogenicity potential in *in vitro* and *in vivo* studies according to OECD guidelines. *Ocimum tenuiflorum* extracts did not cause genotoxicity effects based on micronucleus findings from bone marrow analysis in Sprague-Dawley rats treated up to 5 g/kg bw/day for 7 days [75]. Similarly, extracts were negative in an Ames assay and *in vitro* micronucleus and chromosome aberration tests [76].

4.4. Panax quinquefolius (American Ginseng)

Ginseng is a term often used to cover a number of distinct botanical species in the Genus *Panax*. The two most popular species are *P. ginseng* (Asian ginseng) and *P. quinquefolius* (American ginseng). Both species are used in Traditional Chinese Medicine; however, it is American ginseng that is used to reduce stress, enhance clarity, wisdom, and calmness. The primary phytochemical constituents of ginseng species are the triterpene saponins known as ginsenosides [77]. Ginsenosides are divided into three groups based on chemical structure: 1) Panaxadiols; 2) Panaxatriols; and 3) Oleanolic acids. American ginseng typically contains 4-6% (w/w) total gensenosides [78]. In the last decade, a standardized extract of *P. quinquefolius* L. roots known as Cereboost™ (Naturex; Avignon, France) has become commercially available. Cereboost™ has an enriched ginsenoside content, standardized to 10-12% total ginsenosides [78] and has been developed specifically for its activity on brain function including working memory and alertness [79].

4.4.1. Panax quinquefolius Clinical Efficacy

Prior to the development of standardized extracts like Cereboost™, most clinical studies focusing on cognitive

function were conducted with Asian ginseng (*P. ginseng*). In 2014, Smith *et al.* provided a review of the cognitive effects of ginseng in clinical studies of subjects who were either healthy or suffered from various neurological disorders such as Alzheimer's and dementia [77]. Out of the 17 studies reviewed by Smith *et al.*, only two were conducted with American ginseng (*P. quinquefolius*). These two studies, along with a more recent study conducted with the standardized American ginseng extract; Cereboost™, are briefly discussed below.

Scholey *et al.*, studied the acute effects of American ginseng on mood and neurocognitive function in a randomized, double-blind, placebo-controlled, crossover study in 32 healthy young adults [80]. Three doses of Cereboost™ (100, 200, 400 mg standardized to 10.65% ginsenosides) were used, and subjects were assessed 1, 3, and 6 hours post-dose. Cognitive measures were conducted using the Computerized Mental Performance Assessment System battery which covers the major cognitive domains of attention, working memory, secondary memory and executive function. Mood was assessed *via* the B-L VAS incorporated into the cognitive battery. In summary, the results showed a significant improvement of working memory performance. Choice reaction time accuracy and 'calmness' were significantly improved at the 100 mg dose. Since *P. quinquefolius* has also been shown to have a significant effect on blood glucose in humans and animal models, blood glucose was also monitored in this study, but no changes were observed at any dose level. The authors noted that this was the first study to demonstrate cognitive and mood enhancement with Cereboost™ administration.

The study design discussed above was partially replicated more recently by Ossoukhova *et al.*, in which 200 mg Cereboost™ was given to 52 healthy middle-aged adults (40-60 years old) in a double-blind, placebo-controlled, balanced, crossover study [78]. In addition to using the Computerized Mental Performance Assessment System battery, the CDR battery was also included. Similar to the study by Scholey *et al.*, a single 200 mg dose of Cereboost™ (standardized to 11.65-11.67% ginsenosides) was given to subjects followed by cognitive function testing at 1, 3, and 6 hours post-dose [80]. An acute dose of 200 mg Cereboost™ was shown to improve cognitive performance on working memory at 3 hours post-dose; but had no significant effects on mood or blood glucose levels. These results, which are similar to those shown previously by Scholey *et al.*, indicated that this standardized extract of American ginseng may have some positive effects on working memory [78, 80].

A chronic study (4 weeks) in patients with schizophrenia was conducted with another standardized extract of *P. quinquefolius;* HT1001™ [81]. This was a double-blind, placebo-controlled study in which 64 subjects with schizophrenia were treated with 100 mg (one capsule) of HT1001™ twice daily. Each 100 mg capsule of HT1001™ is equivalent to 500 mg of dried American ginseng root. Working memory (both verbal and visual) was assessed using the Letter-Number Span Test of the WAIS-III). Treatment with HT1001™ for 4 weeks significantly improved visual working memory.

The only mention of adverse event monitoring occurred in the study by Ossoukhova *et al.* In this study, tolerability was assessed *via* monitoring of unsolicited adverse events [78]. Only two participants withdrew from the study; one for reasons unrelated to the study and the second due to an adverse event that was deemed remotely related to the study product (with no explicit description of the adverse event). In a systematic review of 57 clinical trials with ginseng species (American and Asian) by Lee and Son, side effects associated specifically with *P. quinquefolius* included mild insomnia, headache, chest discomfort, and diarrhea [82]. A systematic review of published clinical trials with *Panax* spp. to treat fatigue found a low risk of adverse events associated with the use of either species of ginseng [83].

The safety and tolerability of a patented standardized American ginseng extract has even been studied in children as young as 3 years of age who were suffering from upper respiratory tract infection [84]. The standard dose of extract based on adult weight (70 kg) was 26 mg/kg bw/day on day 1 (maximum 1800 mg), 17 mg/kg bw/day on day 2 (maximum 1200 mg), and 9 mg/kg bw/day on day 3 (maximum 600 mg). A low dose arm was also included which was half the above doses given over the same regimen. The authors concluded that ginseng was well-tolerated, and no serious adverse events were reported. Since *Panax* spp. have been shown in numerous studies to decrease plasma glucose due to improvement of insulin sensitivity, a cautionary statement related to consulting a healthcare practitioner prior to using in diabetics may be warranted [85].

4.4.2. Panax quinquefolius Non-clinical Safety

From a toxicology standpoint, American ginseng is not nearly as well studied as Asian ginseng, with few well-documented toxicity studies available in the literature. No acute or chronic toxicity studies were identified for *P. quinquefolius* based on our recent literature search. There are a few sub-acute and sub-chronic studies that have been performed; however, not enough details of the study designs and/or the botanical preparations used were available to reliably use. For example, in one study, American ginseng root extract or heat-treated root extract was given to male Wistar rats by oral gavage (0 or 100 mg/kg bw/day) for 15 days [86]. At the conclusion of 15 days of dosing, urine and blood was collected for assessment of serum glucose, total protein, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, urea nitrogen, and creatinine. No decrease in renal or hepatic function was observed based on analysis of urine and blood samples from this study.

There is evidence that *P. quinquefolius* root extracts or several of the main phytochemical constituents may have some effect on reproductive endpoints. A 28-day study of orally administered 100 mg/kg bw/day powdered root extract "enhanced" mounting behavior of male rats [87]. Uterine weights were not affected when alcohol extracts of *P. quinquefolius* were administered by oral gavage to ovariectomized CD-1 mice for four days [88]. Some interaction of American ginseng with estrogen receptors (both ERα and ERβ) has been reported; however, results may be dependent on the nature of the extracts and/or which cell lines were used [89-91]. On the other hand, estrogenic activity from

ginseng extracts may be related to contaminants such as mycotoxins. Gray *et al.*, showed that the primary ginsenosides from American and Asian ginseng, Rb1 and Rg1 had no binding to the ER [92]. Analysis of the extracts revealed the presence of zearalenone, the estrogenic mycotoxin produced by several *Fusarium* species. This highlights the role that contamination in botanical raw materials may play in toxicity results.

In a more recent study, alcoholic and aqueous American ginseng root extracts or ginsenosides Rb1, Rg1 and Re alone and in combination were tested for effects on preimplantation development *in vitro* using two-cell mouse embryos [93]. Additionally, the authors studied the effects of whole extracts on pregnancy and post-partum development in mice (up to 2000 mg/kg bw/day) for 2 weeks prior to mating and throughout gestation. American ginseng oral doses did not significantly affect fertility or pregnancy in the mouse, although maternal weight gain was decreased at 2000 mg/kg bw/day). Both alcoholic and aqueous extracts treatment reduced development in a concentration-dependent manner *in vitro*. Only detrimental effects with alcoholic extracts were reversible. Consideration by manufacturers of dietary supplements containing *Panax* spp. to use caution statements on product labels related to avoidance during pregnancy and lactation may still be warranted.

American ginseng root extracts and various phytochemical constituents have been tested in a number of assays to determine genotoxicity potential. No mutagenic activity was detected from either constituent or extracts of *P. quinquefolius* evaluated in Ames assays [94]. These findings are consistent with overall negative findings for Asian ginseng in various genotoxicity assays and in a 2-year bioassay for carcinogenicity conducted by the NTP [95].

4.5. *Centella asiatica* (Gotu Kola)

Centella asiatica (Linn.) Urban (CA) from the family Apiaceae, commonly known as Gotu kola has been used traditionally as both food and herbal medicine. It is one of the most commonly consumed leafy green vegetables in many South East Asian countries. It is considered to have great nutritional benefits as it contains carotenoids, vitamin B and C, minerals, and other phytonutrients including flavonoids and polyphenols. The medicinal benefits of Gotu kola have included antidiabetic, wound-healing, antimicrobial, memory enhancing, antioxidant, and neuroprotecting properties. Specific to neurological properties, it has been proposed to help with fatigue, anxiety, depression, memory improvement and cognitive function [96].

4.5.1. *Centella asiatica* Clinical Efficacy

Unfortunately, robust human clinical trials to study the many purported benefits and nutritional values of *C. asiatica* are limited, and this has perhaps hindered its more global use. A recent systematic review and meta-analysis of clinical efficacy with *C. asiatica* on cognitive function found no significant improvement with use of this botanical [97]. However, the authors concluded that *C. asiatica* may improve working memory and mood based on self-reported alertness and reduction in self-reported feelings of anger. The positive outcome on working memory was studied in the randomized,

placebo-controlled, double-blind trial conducted by Wattana-thorn *et al.*, [98]. Twenty-eight healthy elderly subjects were given 250, 500, or 750 mg once daily doses of *C. asiatica* extract (asiaticoside and asiatic acid content 1.09 and 48.89 mg/g of crude extract; respectively) for 2 months. Cognitive performance was assessed using a modified CDR computerized test battery and event-related potential (N100 and P300 amplitude and latencies), and mood was assessed using the B-L VAS. Results showed that the high dose plant extract enhanced working memory and mood in healthy elderly, and thus could decrease age-related decline in cognitive function.

The major phytochemical constituents that are thought to be associated with various activities are asiaticoside and madecassoside, and respective aglycones (asiatic acid and madecassic acid). Taken together, these phytochemical constituents are known as centellosides. There are two well-characterized and standardized extracts of *C. asiatica* that are commercially available. ECa 233 is a standardized *C. asiatica* extract developed in Thailand that contains >80% triterpenoid glycosides with a constant proportion of madecassoside and asiaticoside at 1.5 ±0.1:1 [99]. The standardized extract of CA (L.) Urban (INDCA) is made with dried *C. asiatica* leaves that are extracted with isopropyl alcohol (1:5), filtered, concentrated, washed with a variety of solvents and then freeze dried. It is then standardized for asiaticoside content [100].

Despite the availability of these standardized extracts there is still a paucity of human clinical efficacy and safety data. The pharmacokinetics and tolerability of 250 and 500 mg of ECa 233 were recently studied in healthy Thai volunteers [99]. The study was an open-labeled, 2 sequence dosage, single- and repeated-dose study that was conducted under fasted conditions. Plasma concentrations of asiaticoside and madecassoside and respective metabolites, asiatic acid and madecassic acid, were monitored. Tolerability was assessed based on physical examinations, monitoring of vital signs, clinical chemistry (hematology, electrolytes, kidney and liver function tests), and observation of adverse events. The Cmax and AUC of asiaticoside and madecassoside were very low, while the acid metabolites of both were much higher (Table **4**). Furthermore, this study indicated that the accumulation of active metabolites after repeated doses appears likely. The asiatic acid profile showed a 2-fold increase in Cmax and $AUC_{(0-t)}$ after increasing the dose from 250 to 500 mg of ECa 233.

ECa 233 was considered safe and well-tolerated at both dose levels and with both single- and repeated dosing. There were no serious adverse events reported. Only mild to moderate adverse events were reported in 5 of 11 subjects, and the 6 events that were considered ECa 233-related included moderate drowsiness (n=3), mild stomach irritation (n=1), moderate frequent urination (n=1), and moderate headache (n=1). Most importantly, all adverse events remained only in the single-dose study and did not occur with repeated dosing. There were no significant changes in any other safety parameter studied including clinical chemistries.

The findings of safety and tolerability for ECa 233 are consistent with findings in various short-term clinical studies with non-standardized *C. asiatica* extracts. Occasional com-

Table 4. Summary of key human pharmacokinetic parameters determined for major bioactive compounds and metabolites of standardized extract of *C. asiatica,* ECa 233 (as abbreviated from Songvut *et al.*) [99].

	Madecassoside				Asiaticoside			
	Day 1		Day 7		Day 1		Day 7	
PK Parameters	250 mg (N=11)	500 mg (N=10)	250 mg (N=11)	500 mg (N=10)	250 mg (N=11)	500 mg (N=10)	250 mg (N=11)	500 mg (N=0)
	A - Parent bioactive compounds (Madecassoside and Asiaticoside)							
Cmax[a] (µg/L)	3.55 ± 1.79	5.67 ± 0.62	5.75 ± 3.17	5.23 ± 1.84	1.043 ± 0.14	1.50 ± 0.18	5.93 ± 21.20	2.71 ± 1.08
Tmax[b] (h)	1 [2]	1[1]	2[2]	1[0]	1[1]	1[1]	2[0]	2[0]
AUC[a] (µg*h/L)	12.43 ± 6.70	30.12 ± 7.12	22.1 ± 11.68	37.29 ± 16.52	1.0 ± 0.23	1.70 ± 0.53	10.8 ± 10.18	11.40 ± 9.67
	B - Active metabolites (Madecassic acid and Asiatic acid)							
Cmax[a] (µg/L)	40.92 ± 25.78	52.14 ± 18.67	42.71 ± 21.06	80.79 ± 27.76	38.02 ± 12.21	84.08 ± 33.91*	51.28 ± 17.91	116.62 ± 32.26*
Tmax[b] (h)	2[1]	1.5[1]	1[1]	1.5[1]	1[7]	1[0]	1[1]	1[0]
AUC[a] (µg*h/L)	348.44 ± 362.47	357.20 ± 116.65	412.66 ± 375.88	681.05 ± 413.17	434.13 ± 195.72	724.75 ± 259.98*	624.97 ± 277.14	1202.29 ± 293.37*

[a]Data expressed as mean ± standard deviation; [b]Data expressed as median (IQR); *$p<0.05$ for significant differences; Cmax = maximum plasma concentration; Tmax = time to reach Cmax; AUC = area under the plasma concentration-time curve from time zero to the last measurable concentration.

plaints reported across these studies have included minor gastric issues and nausea, but usually not significant when compared to placebo [101].

4.5.2. Centella asiatica Non-clinical Safety

From a toxicology standpoint, there are well-executed sub-chronic rat oral toxicity studies on both standardized extracts of *C. asiatica* (ECa 233 and INDCA). The NOAELs from these studies can be used to support chronic oral exposure to the standardized extracts. For powdered plant or non-standardized extracts that may have different phytochemical constituent profiles compared to INDCA and ECa 233, additional toxicity studies may need to be conducted.

A 90-day rat oral sub-chronic study with ECa 233 was conducted at dosages of 0, 10, and 100 mg/kg bw/day [102]. There were no biologically relevant changes in body weight, food consumption or behavior. There were some significant changes in hematology parameters for the treated females, but no dose-response was observed. For the clinical chemistry parameters measured, the only significant change was higher sodium in the high dose males. Low dose males had significantly lower adrenal weights compared to controls. There were no differences noted in gross pathology exams. Low dose males also had a significantly high incidence of lymphoid proliferation in the lung and centrilobular fatty degeneration in the liver compared to controls. These changes were not observed in the mid or high dose animals. A NOAEL for ECa 233 in this study was established at 1000 mg/kg bw/day.

Deshpande *et al.*, conducted a rat sub-chronic oral toxicity study with INDCA according to OECD 408 [100]. Spra-

gue-Dawley rats received distilled water (15 rats/gender + 10/gender for a 20-day recovery), 250mg INDCA/kg bw (15rats /gender), 500mg INDCA/kg bw (5 rats/gender), 1000 mg INDCA/kg bw (15 rats/gender + 10/gender for a 20-day recovery). There were very few signs of toxicity in any of the parameters measured. Ophthalmic and functional observational tests were also conducted. There were no significant changes in food consumption or body weight gain in treated versus control rats. Hematology and biochemical parameters did have some significant changes in treatment group compared to controls, but the effects were not dose-dependent. Urinalysis results, organ weight data and gross pathology data were similar across all groups. Histopathological examination revealed some minimal to mild changes in the liver (focal lymphocytic infiltration and/or focal necrosis) and kidneys (minimal focal to multifocal lymphocytic infiltration, necrosis and/or hydronephrosis) in both the controls and high dose treatment group. The authors considered the NOAEL from this study to be 1000mg/kg bw/day.

Mixed results have been published on the reproductive toxicity of *C. asiatica* extracts using non-standard animal study protocols. Extracts of *C. asiatica* (most likely concentrated) have been reported to be toxic to the reproductive system of male rats [103, 104]. However, other authors have tested dilute *C. asiatica* extracts and reported a lack of effect at similar dose levels [105]. In addition, the two sub-chronic oral toxicity studies described above did not find any evidence of potential reproductive organ toxicity. The primary metabolite, asiatic acid, did not have an adverse effect on the testis as reported by Maio *et al.,* [106]. As a result of these conflicting results and the lack of data from definitive toxicology studies to assess the effects of *C. asiatica* on devel-

opmental and reproductive endpoints, the botanical should be avoided during pregnancy. This guidance is consistent with current European Medicines Agency (EMA) and European Scientific Cooperative on Phytotherapy (ESCOP) cautions [101, 107].

An aqueous-ethanol extract of *C. asiatic*a and INDCA extract have both tested negative for mutagenicity in Ames assays [100, 108]. An acetone leaf extract of *C. asiatica* was not clastogenic when evaluated in the chromosomal aberration assay using human peripheral blood lymphocytes [109].

4.6. *Melissa officinalis* L. (Lemon Balm)

Lemon balm (*Melissa officinalis* L.) has a long history of use dating to ancient Greek and Roman medicine [110]. Traditional uses for lemon balm include relief of mild symptoms of mental stress and insomnia, and for symptomatic treatment of mild gastrointestinal complaints. In general, the modern use of lemon balm is supported by a long history of use, and the EMA concluded that it meets the EC Directive 2004/24 requirement of 30 years in medical use to qualify as a traditional herbal medicine for several preparations [111]. Additionally, lemon balm is used for flavoring food, as tea preparations, and as a fragrance [111-113]. The FDA lists *Melissa officinalis* L. as Generally Recognized as Safe (GRAS) for spices and other natural seasonings and flavorings, as well as for essential oils, oleoresins (solvent-free) and natural extractives, including distillates for intended use in food for human consumption [114].

We evaluated a total of 5 clinical efficacy studies related to sleep, anxiety, stress, or cognition endpoints. The studies described herein were chosen as they were considered of the highest quality and robustness in evaluating the stated efficacy endpoints. Two recently conducted clinical studies have been conducted on the ability of *M. officinalis* to relieve anxiety and promote sleep. A brief review of two additional studies with non-standardized extracts of *M. officinalis* have also been included as they did assess safety parameters in a clinical study. Details of these clinical trials are provided below and summarized briefly in Table **5**.

4.6.1. *Melissa officinalis* Clinical Efficacy

Soltanpour and colleagues studied the effects of *M. officinalis* dried leaf powder on quality of sleep and in relieving anxiety in a randomized, double-blind, placebo-controlled study [115]. Eighty volunteer in-patients who were undergoing coronary artery bypass surgery were given 500 mg capsules of *M. officinalis* dried leaf powder three times a day (morning, noon, and before sleep) for seven days. Sleep outcome was assessed using the St. Mary's Hospital Sleep Questionnaire; an instrument that has been shown to be valid and reliable in numerous studies. The anxiety outcome was evaluated using a version of the HADS. The reliability and validity of HADS have also been confirmed previously. The anxiety scores were significantly improved in the *M. officinalis* treatment group over placebo (P=0.001); and the mean changes of sleep quality were also significantly higher with treatment (p<0.001). The authors concluded that 1.5 g/day of dried leaf powder of *M. officinalis* appeared to reduce the levels of anxiety (by 49%) and improve sleep quality (by 54%) in patients undergoing coronary artery bypass surgery. Furthermore, treatment with *M. officinalis* was well-tolerated with no reports of adverse effects.

A second, high quality study was conducted in children experiencing sleep bruxism (SB) who were treated with either *M. officinalis* (leaves or stalks) or *Phytolacca decandra* (roots of poke weed) alone or in combination [116]. The study was a randomized, triple-blinded, placebo-controlled cross-over study in 52 children ages 3 to 12 years (average age 6.62 ± 1.79 years). The botanical treatments were homeopathic medicines and were provided as ethanolic mother tinctures following the Brazilian Homeopathic Pharmacopoeia [117]. The profiles of a number of phytochemical constituents were analyzed by liquid chromatography with diode array and mass spectrometry, and a number of phytochemical constituents were quantitatively monitored.

The children were treated with one drop per age in 15 mL of water daily, 20-30 minutes before bedtime for 30 days. The primary outcome was measured by parents as a value in a VAS which included millimeter degrees and stages of tooth grinding. The VAS ranged from 0-10 cm and was divided into three stages of tooth grinding: light (0 to 2.9 cm), moderate (3.0 to 7.9 cm) and intense (8.0 to 10.0 cm). A sleep diary was also used to record the parent's perception of the children's sleep routine. A dental technician also applied the Trait-anxiety questionnaire to the parent before each treatment phase to measure the level of anxiety in the children suffering from sleep bruxism. Lastly, physical adverse effects were also investigated by questioning parents as to any observed changes in the health of their children during treatment.

A significant reduction in SB was observed based on VAS across all treatment groups, including placebo; however, the *M. officinalis* alone treatment leg showed better results compared to *P. decandra* (P=0.018) and placebo (p=0.050), and comparable to the combination of *M. officinalis* and *P. decandra* (P=0.724). The sleep diary results and Trait-anxiety questionnaire were not significantly different across any of the treatment groups. No side effects of treatments were noted by parents. Although this study used homeopathic preparations of *M. officinalis*, it did show promising results in treating a disorder related to anxiety.

The final series of studies included one pilot and two randomized, placebo-controlled, balanced crossover clinical studies to examine mood, cognitive and anti-stress effects after treatment with Lemon balm (*M. officinalis)* [118]. These studies were unique in that the lemon balm extract was formulated into foods, including an iced tea drink and yoghurt product. The lemon balm water extract was prepared from dried leaves and standardized to >2% rosmarinic acid. The small-based pilot study (N=5) was conducted on healthy subjects who considered themselves to lead a stressed lifestyle. Subjects fasted for 10 hours prior to visiting the treatment facility, where they were evaluated using the CDR core battery; a computerized cognitive assessment system. This battery covers the cognitive domains of attention/concentration, short term working memory and long-term secondary memory. Mood was also assessed using a computerized version of the B-L VAS questionnaire. Additionally, two traditional,

Table 5.   Summary of efficacy, pharmacokinetic, safety and tolerability clinical studies with *Melissa officinalis* (Lemon balm) extracts.

Refs.	Study Design	Endpoints	Dosage & Duration	Results
[115]	RDBPC[a]	Sleep and anxiety	1500 mg/d (500 mg three times daily) *vs.* placebo X 7 days	*M. officinalis* appeared to reduce the levels of anxiety (by 49%) and improve sleep quality (by 54%) in patients undergoing coronary artery bypass surgery.
[116]	RTBPC[b], cross-over	Sleep bruxism	Homeopathic tinctures (1 drop per age in 15 mL of water daily *M. officinalis*, *P. decandra*, or placebo) X 30 days	Significant reduction in sleep bruxism observed across all treatment groups including placebo, *M. officinalis* treatment showed better results than *P. decandra* or placebo.
[118]	Pilot study	Determine bioavailability of rosmarinic acid from lemon balm extract, establish time points for assessing cognition and mood	-	Rosmarinic acid was bioavailable with peak plasma levels achieved within 1 hour of dose, returning to baseline between 4-6 hours post-dose.
[118]	RPC[c], balanced cross-over	Cognition, mood	480 mL 0.3 g lemon balm + natural fruit sweetener, 0.6 g lemon balm + natural fruit sweetener, 0.6 g lemon balm + blend of artificial sweeteners, or placebo	0.3 g lemon balm + natural fruit sweetener associated with lower anxiety and better working memory at both 1 and 3 hours post-ingestion
[118]	-	Cognition, mood	250 mg lemon balm in yoghurt	Indications of benefits from 0.3 g dose including improvement in alertness and word recall at 1 hour post-dose and math performance improving at 3 hours. The 0.6 g lemon balm-yoghurt formulation associated with more fatigue even at 1 hour post-dose.
[119]	RPC	Pharmacokinetic, safety, and tolerability	*M. officinalis* treatments containing 100 mg, 250 mg, 500 mg rosmarinic acid, placebo	No detection in plasma with 100 mg rosmarinic acid in 4 of 6 participants, Tmax of rosmarinic acid at 1 hour post-dose with 250 mg or 500 mg rosmarinic acid. Cmax of 72.22± 12.01 nmol/L and 162.20 ± 40.20 nmol/L after 250 mg and 500 mg rosmarinic acid; respectively. Food intake increased AUC by 1.3 times, and delayed Tmax. M. officinalis containing 500 mg rosmarinic acid did not affect blood chemistries.
[120]	RDBPC	Safety, efficacy (diabetes endpoints)	700 mg *M. officinalis* extracts daily X 12 weeks	No safety related treatment effects
[121]	RDPBC	Safety, efficacy anxiety (and cardiovascular endpoints)	1000 mg daily (500 mg twice daily) X14 days *M. officinalis* extract *vs.* placebo	No indication of side effects, significantly reduced anxiety (and frequency of heart palpitations)

[a]Randomized, double-blinded, placebo-controlled. [b]Randomized, triple-blinded, placebo-controlled. [c]Randomized, placebo-controlled.

validated written questionnaires were used to assess mood; the POMS and the Spielberger State Anxiety Questionnaire. Blood samples were also taken at baseline, 30 minutes, and 2, 3, 4, 6, 8, and 12 hours post-consumption of the test food to monitor for rosmarinic acid as a biomarker of bioavailability.

In the first clinical study, twenty-five healthy subjects ranging in age from 18 to 39 years of age were treated with a similarly prepared lemon balm extract albeit with a >6% rosmarinic acid content. Likewise, the standardized lemon balm extract was provided to study subjects in the form of an iced-tea drink. Subjects were given 480 mL of either 0.3 g lemon balm + natural fruit sweetener, 0.6 g lemon balm + natural fruit sweetener, 0.6 g lemon balm and a blend of artificial sweeteners, or a placebo beverage containing a blend of artificial sweeteners. After a practice session, the subjects visited the testing laboratory on four additional occasions to perform the various tasks. During each visit, subjects completed three blocks of testing with each block consisting of a State-Trait Anxiety Inventory, saliva sample, word presenta-

tion, immediate word recall, B-L VAS (stress and fatigue), 20 minute MTF, B-L VAS, delayed word recall, word recognition, saliva sample, and State-Trait Anxiety Inventory. At the end of the last block, participants completed a symptom checklist.

The second clinical study included the same standardized lemon balm extract as used previously; but delivered as a 250 g yoghurt product. Twenty-one subjects ages 21 to 30 years were given the same doses of lemon balm and submitted to the same testing design as in the first clinical study. The testing and outcome measures were identical to the previous clinical study, with minor exceptions. The Bond-Lader mood scales used in the second study were a pencil-and-paper version and the Stress VAS was also a pencil-and-paper version which included an additional Fatigue VAS ("How mentally fatigued do you feel right now?").

The initial pilot study provided evidence that rosmarinic acid from lemon balm delivered as an iced-tea drink was bioavailable, with peak plasma levels achieved within 1 hour of consumption and returning to baseline between 4 and 6 hours. The cognitive and mood data collected across various time points in the pilot leg helped establish appropriate time points to assess these parameters in the two follow-on clinical studies.

Data from the two clinical studies indicated that lemon balm treatments were generally associated with improvements in mood and/or cognitive performance. In the first clinical study, the iced-tea drink containing 0.3 g lemon balm and natural fruit sweetener was associated with lower anxiety and better working memory at both 1- and 3 hours post-ingestion, indicating potential for anxiolysis over a sustained period. In fact, this effect is similar to what is observed with benzodiazepines; without the negative impairments on performance (*e.g.*, psychomotor). Working memory performance was also significantly improved by the same treatment at 1- and 3 hours post-ingestion. Higher doses of lemon balm (0.6 g) improved mathematical processing at 1 hour only and significantly improved psychomotor performance as assessed by the tracking module. Interestingly, the 0.6 g lemon balm iced-tea drink with artificial sweeteners resulted in a higher state of anxiety at 3 hours post-drink. The authors associated this effect with the presence of artificial sweeteners. The anxiolysis effects of lemon balm tracked with measured cortisol responses in subjects.

The effects in the second clinical study with lemon balm administered *via* yoghurt preparations were less straightforward. There were indications of benefits from the lower (0.3 g) dose including improvement in alertness and word recall at 1-hour post-consumption and math performance improving at 3 hours. However, the 0.6 g lemon balm-yoghurt formulation was associated with more fatigue even at 1-hour post dose. The authors noted other differences in the results of the two preparations. Working memory (where information is held "online" in consciousness) was improved by 0.3 g lemon balm at both 1 and 3 hours in the iced tea drink. Yoghurt preparations including both 0.3 g and 0.6 g lemon balm were associated with better word recall (declarative memory involving encoding and retrieval of information).

A randomized placebo-controlled clinical trial to assess pharmacokinetics, safety and tolerability of *M. officinalis* extract enriched for rosmarinic acid content was conducted in eleven healthy individuals [119]. The primary outcome of the study was to investigate the pharmacokinetics of rosmarinic acid, including determining whether there was a food effect in humans. Secondary outcomes were related to safety and tolerability as measured by clinical and physical examinations, recorded adverse events, and laboratory blood chemistry measurements, including hematology (white blood cell count, red blood cell count, hemoglobin levels, hematocrit and platelet count), and blood biochemistry (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, total bilirubin, blood urea nitrogen, and creatinine). All parameters were measured at baseline and 48 hours after the single dose *of M. officinalis* containing rosmarinic acid.

There were three possible *M. officinalis* treatment legs containing either 100 mg, 250 mg, or 500 mg rosmarinic acid. Each study participant received 3 interventions (placebo, 100 mg, 250 mg, or 500 mg rosmarinic acid) using a Latin square design and each dose separated by a 10-day wash-out period. In the initial study, participants were fasted prior to administration of treatment and maintained a low polyphenol diet during the course of the study. In the second study, participants were fed a low polyphenol meal before dosing.

In this study, plasma concentration of total rosmarinic acid peaked at 1 hour after administration of *M. officinalis* containing 250 mg or 500 mg of rosmarinic acid; there was no detection in plasma with 100 mg rosmarinc acid in 4 of 6 subjects. The maximum concentration of rosmarinic acid in human plasma was 72.22 ± 12.01 nmol/L (mean ± SEM) and 162.20 ± 40.20 nmol/L after 250 mg and 500 mg rosmarinic acid; respectively. Food intake increased the area under the curve by 1.3 times, and delayed time to reach maximum plasma concentration. Furthermore, single doses of *M. officinalis* containing as much as 500 mg rosmarinic acid did not affect any laboratory blood chemistry endpoints, and no adverse events were recorded.

Safety parameters were also included in at least two other recently conducted clinical studies primarily designed to assess various efficacy endpoints associated with *M. officinalis* treatment in patients with either diabetes or suffering from benign heart palpitations [120, 121]. Although these studies measured various clinical chemistry parameters and recorded adverse events, the *M. officinalis* extracts were not standardized.

In the study by Asadi *et al.* the subjects who were treated with 700 mg hydroalcoholic extract of *M. officinalis* received 8.10 ± 0.04 mg rosmarinic acid per 350 mg capsule (~16 mg rosmarinic acid total) per day for 12 weeks [120]. The authors reported a lack of significant effects of treatment on aspartate and alanine aminotransferases, and alkaline phosphatase, and no other adverse events. Alijaniha *et al.*, treated volunteers with 500 mg of a lyophilized aqueous extract of *M. officinalis* leaves for 14 days [121]. No significant treatment-related effects were recorded in complete blood

counts, serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, blood urea nitrogen, and creatine levels. The only significant treatment-related effect reported was an "increased appetite" in the *M. officinalis* treatment group. Unfortunately, no phytochemical constituent characterization data or standardization of extracts was provided.

4.6.2. Melissa officinalis Non-clinical Safety

No standard or guideline (*e.g.*, OECD) toxicology studies have been conducted on *M. officinalis* in animals. Hashemnia *et al*. conducted a 30-day repeat dose toxicity study in Sprague-Dawley rats [122]. Animals were treated by oral gavage with either 600 or 1200 mg/kg bw/day of a hydroethanolic extract of *M. officinalis* leaves, or saline placebo. The extracts were not standardized to any phytochemical constituents, and no characterization of any constituents was conducted. The study did include 10 animals per treatment group; however, no description of whether both males and females were included. Toxicology endpoints included in-life observations, body weight, food and water consumption, hematology, serum chemistry, and histopathological examination of only kidney, liver, spleen, heart and lung. There was no recovery leg included.

At the conclusion of this study, all animals showed an overall increase in body weight, although the higher dose group showed a lower average body weight increase. No significant change in most hematological parameters was noted; however, red blood cell counts were decreased in both treatment groups, and clear leukocytosis was also recorded, but not statistically significant from control group. Histopathologic lesions indicated mild to moderate liver injury (hepatocyte degeneration, congestion and dilation of sinusoids, proliferation of bile ducts, polymorphic hepatocytes and infiltration of mononuclear cells around the portal area); which was consistent with an increase in alanine aminotransferase with treatment of *M. officinalis*. There was also mild to moderate kidney histopathology based on tubular degenerations and necrosis, tubular and glomerular atrophy and congestion in treated rats, which was also consistent with a significant increase in creatinine concentration. Since the majority of these findings occurred at both dose levels, a NO(A)EL was not established in this study.

The genotoxicity potential of lemon balm has been evaluated to some extent in various test systems such as the Ames assay (mutagenicity), and *in vitro* micronucleus and comet assays (clastogenicity). These studies were not conducted according to current international guidelines; however, in no cases were there any sign of positive findings. There have been no studies conducted to understand the effects of lemon balm on developmental and/or reproductive toxicity endpoints. Conclusions regarding safe use of lemon balm during pregnancy and lactation vary from prohibitive statements [123] to "unlikely problematic," [124].

4.7. Salvia officinalis (Common sage) and Salvia lavandulaefolia (Spanish Sage)

Salvia officinalis is a perennial plant native to the Mediterranean region, and the leaf plant part has been used for centuries dating back to ancient times as part of Egyptian,

Greek, and Roman medicines [110]. *Salvia lavandulaefolia* (Spanish sage) is native to Spain and southern France. *Salvia officinalis* has traditionally been regarded for its carminative (gas prevention), antispasmodic, antiseptic, astringent, and antihidrotic (sweat prevention) properties. *Salvia officinalis* (and *S. triloba*) are FDA GRAS as essential oils, oleoresins (solvent-free), and natural extractives (including distillates) [114].

4.7.1. Salvia officinalis and Salvia lavandulaefolia Clinical Efficacy

Various species of the genus *Salvia* (sage) have been used to promote healthy brain function in traditional medicine practice. The most commonly used species of *Salvia* in clinical studies aimed at studying effects on cognition are *S. officinalis* (common sage) and *S. lavandulaefolia* (Spanish sage). Two independent reviews of the potential of various *Salvia* species to enhance cognitive endpoints, including dementia were recently published [125, 126]. Both reviews summarized the same clinical studies associated with the efficacy of *S. officinalis* and *S. lavandulaefolia*; and both concluded that there was evidence to suggest that sage could improve cognitive function and alertness. Our more recent literature search did not yield any new clinical efficacy or safety studies since these 2017 reviews. Thus, only a description of the human safety and toxicity of *Salvia* spp. will be discussed below.

The doses of *S. officinalis* and *S. lavandulaefolia* used in the studies referenced above were well tolerated with no; or limited adverse events. In the study by Akhondzadeh *et al*., in which patients with mild to moderate Alzheimer's disease were treated with *S. officinalis*, 6 cases of side-effects were observed over the course of the 4-month trial [127]. The side effects included vomiting, dizziness, wheezing, agitation, abdominal pain, and nausea. The frequency of these effects was not significantly different between placebo and treatment; with the exception of agitation which was higher in the placebo group.

Although only a pilot open-label study, Perry *et al*., conducted extensive safety monitoring of subjects with Alzheimer's disease before and after taking *S. lavandulaefolia* essential oil capsules for 6 weeks [128]. In addition to recording adverse events, blood samples were screened for a number of hematological and clinical chemistry endpoints. No subjects experienced any adverse physical or neurological effects, or no statistically significant changes in any blood sample parameter at the completion of the study. An increase in bilirubin after 6 weeks of treatment with *S. lavandulaefolia* was measured; however, this was not statistically significant. The study sponsors concluded that treatment of Alzheimer's patients with *S. lavandulaefolia* essential oil for 6 weeks was well-tolerated and resulted in a statistically significant reduction in neuropsychiatric symptoms and an improvement in attention over baseline measurements.

4.7.2. Salvia officinalis and Salvia lavandulaefolia Non-clinical Safety

There is a lack of high-quality toxicology studies that have been conducted with *Salvia* spp. Thus, long-term oral

Table 6.   **Details of key phytochemical constituents (%) present in essential oil of *Salvia officinalis* obtained from different regions.**

Constituent	Turkey	Jordan	US	Iran	Iran	Tunisia	Brazil	Albania North/South
α-thujone	20.6	1.2-3.7	25.8	33.72	-	13.45	24.8	30.72/15.92
B-thujone	15.1	0.1-9.9	5.7	3.87	-	18.40	3.97	5.38/1.71
Camphor	22.9	8.8-25	6.4	2.94	3.28	3.31	10.9	26.57/43.83
References	[132]	[133]	[134]	[135]	[136]	[137]	[138]	[139]

exposure of *S. officinalis* can be supported by current use as a spice or flavor in food up to 930 mg of sage quantity crude equivalent/person/day according to the Possible Average Maximum Daily Intake (PAMDI) in the Research Institute for Fragrance Materials database (FEMA No. 3000) [129]. The PAMDI is the sum of the botanical exposure from multiple food categories based on the maximum level of the botanical in each food category and the average daily intake for each food category. Dietary supplement exposure for *S. officinalis* is typically within the range of the PAMDI. Safe exposure at the PAMDI is also substantiated by the multiple clinical trials that last up to 3 months with a dosage of up to 1500 mg sage leaf extract. *S. officinalis* essential oils, extracts and oleoresins are also listed as US FDA GRAS [114].

In assessing the safety of *Salvia* spp. It is worth considering toxicity potential of some of the major phytochemical constituents. Sage leaf contains at least 1-2% essential oil, of which major constituents include α-thujone (10-60%), β-thujone (4-36%) and camphor (5-20%) [130]. These substances are considered to be toxic at high doses, particularly to the central nervous system (*e.g.*, convulsions). A number of significant adverse events have been documented in humans in conjunction with exposure to sage tea and sage oil and the associated thujone and camphor constituents. The EMA stated that prolonged use of the alcoholic extract or pure essential oil can produce epileptiform convulsions [130]. Clinical intoxications with sage oil have been characterized by tonic-clonic or clonic convulsions and a comatose state, which the EMA indicated was related to the camphor and thujone content of the oil. Ingestion of 0.7 - 1.0 g camphor has been shown to be fatal in children according to EFSA [131].

The levels of these constituents can vary greatly across *Salvia* spp; for example, levels of thujone are reported to be higher in *S. officinalis* compared with *S. lavandulaefolia* [125]. Even within the same *Salvia* spp., levels of these constituents can vary across different geographic regions (Table **6**).

Although no carcinogenicity studies have been conducted on *Salvia* spp., a number of genotoxicity studies have been conducted with *S. officinalis* and constituents including thujone and camphor. In general, the available data suggest that sage leaf preparations or key components possess little to no genotoxic activity [130, 140]. Likewise, data from developmental and reproductive studies in animals conducted according to current guidelines are not available. Many governmental regulatory agencies and other authoritative

sources recommend that sage leaf or sage oil use be avoided during pregnancy or lactations [140, 141].

4.8. *Mentha spicata* L. (Spearmint)

Spearmint, *Mentha spicata* L., family Lamiaceae, is another botanical that is readily consumed in the diet; largely as a flavor ingredient. It is also used in cosmetic products like shampoos, soaps, and toothpastes. Traditionally, spearmint has been used to treat gastrointestinal symptoms, respiratory problems, dandruff, and as a sedative [142]. There are also reports of its use as an abortifacient and emmenagogue. Spearmint has only more recently been studied for the ability to improve cognitive function; probably resulting from its relationship to other members of the Lamiaceae family (*e.g.*, *Salvia officinalis*, *Melissa officinalis*) used for nootropic effects. Rosmarinic acid is a primary phytochemical constituent in botanicals of the Lamiaceae family, and considerably high concentrations may be present in *Mentha* species like *M. spicata*. The neuroprotective properties of phytochemicals like rosmarinic acid may hold great promise in improving cognitive function [143].

4.8.1. *Mentha spicata* L. Clinical Efficacy

The most well-studied spearmint extract in both non-clinical and clinical studies, is a proprietary standardized extract of dried spearmint leaves that contains 15.4% rosmarinic acid, and is supplied by Kemin Foods L.C. (Des Moines, IA). This extract is sourced from two proprietary spearmint plants, grown in the United States according to Good Agricultural Practices, and manufactured using current Good Manufacturing Practices (cGMP) for food (21 CFR 110) [1]. This review of the clinical efficacy and safety, and non-clinical toxicology of *M. spicata* will focus on this well-characterized proprietary spearmint extract (designated PSE below); and results are summarized in Table **7** below.

In an open-label pilot study to assess the tolerability, bioavailability, and potential cognitive function outcomes, 900 mg of the PSE was given to 11 subjects (50-70 years of age) for 30 days [144]. Tolerability was assessed by hematology, plasma lipid profiles, and gastrointestinal symptom questionnaire. Plasma levels of rosmarinic acid and metabolites including vanillic acid sulfate, caffeic acid sulfate, dihydrocaffeic acid sulfate, ferulic acid sulfate, dihydroferulic acid sulfate, and methyl rosmarinic acid glucuronide were monitored 0.5- and 2 hours post-dose. Cognitive function was assessed using a computerized battery of tests (Cambridge Brain Sciences, London, Ontario, Canada). At the

Table 7.   **Summary of efficacy, bioavailability, safety and tolerability clinical studies with a proprietary spearmint extract (PSE) of** ***Mentha spicata*** **L. standardized with 15.4% rosmarinic acid.**

Refs.	Study Design	Endpoints	Dosage & Duration	Results
[144]	Open-label pilot	Tolerability, bioavailability, efficacy (cognitive function)	900 mg X 30 days	PSE was well-tolerated, rosmarinic acid and metabolites detectable in plasma, improvement in computerized cognitive function scores.
[145]	RDBPC[a]	Safety and tolerability	600 mg, 900 mg PSE or placebo daily X 90 days	No effects on any safety parameters measured, no AEs deemed relevant to PSE.
[146]	RDBPC	Cognitive function (performance, sleep, mood)	600 mg, 900 mg, or placebo daily X 90 days	Working memory and spatial working memory accuracy improved by 15% and 9% (900 mg PSE). Subjects reported improvement in ability to fall asleep at 900 mg PSE. Treatment-related trends for vigor-activity and total mood disturbance vs. placebo. The only treatment-related AE was heartburn (1 subject, 600 mg PSE).
[147]	RDBPC, parallel design	Cognition	900 mg daily PSE X 90 days	Significant treatment effects observed for attention (sustained, complex, shifting).

[a]Randomized, double-blinded, placebo-controlled.

conclusion of the studies, the authors reported that the PSE was well-tolerated, rosmarinic acid and several metabolites were detectable in plasma after acute administration, and there was an improvement in computerized cognitive function scores.

A second safety and tolerability study with the PSE was conducted as a randomized, placebo-controlled, double-blind design [145]. This study investigated doses of 600 and 900 mg/day of the PSE for 90 days in healthy adults with age-associated memory impairment. Tolerability assessments included hematology, plasma chemistry (which included measurements of liver and kidney function), hormones (follicular stimulating hormone, luteinizing hormone, and thyroid stimulating hormone), and lipid profiles. Adverse events were self-reported. At the conclusion of the study, there were no effects observed on plasma levels of follicular stimulating hormone, luteinizing hormone, or thyroid stimulating hormone, or any other of the safety parameters measured. There were no reported severe adverse events or any adverse events deemed relevant to the PSE.

The safety and tolerability studies described above set the groundwork for further randomized, controlled clinical efficacy studies with the PSE and cognitive function outcomes. Subjects (N=90) with age-associated memory impairment were given 0, 600, or 900 mg/day of the PSE for 90 days in a randomized, placebo-controlled, double-blind trial [146]. The clinical outcomes were on cognitive performance, sleep and mood using the CDR system, Leeds Sleep Evaluation Questionnaire (LSEQ), and POMS™; respectively. At the conclusion of the study, working memory and spatial working memory accuracy improved by 15% (p=0.0469) and 9% (p=0.0456); respectively with 900 mg/day PSE administration. Subjects reported improvement in ability to fall asleep (p=0.0046 versus placebo) at 900 mg/day dose. There were also treatment-related trends for vigor-activity (p=0.0399) and total mood disturbance (P=0.0374) when compared to placebo. The only treatment-related adverse event was heart-

burn experienced by one subject in the 600 mg/day PSE group.

The potential nootropic effects previously observed with the PSE were further investigated in a randomized, double-blind, placebo-controlled, parallel design in healthy (N=142) subjects (ages 18-50 years) [147]. Cognition was assessed *via* a computerized cognitive test battery (CNS Vital Signs Inc, Morrisville, NC, USA) that included 10 cognitive domains (finger tapping, symbol digit coding, Stroop, shifting attention, continuous performance, reasoning, 4-part continuous performance, and digit span). Mood was assessed by POMS Standard Form questionnaire. Measurements were made on days 0, 7, 30, and 90 days of supplementation with 900 mg of PSE. Quality of life, exercise, and food logs were also assessed. At the conclusion of the study, significant treatment effects were observed for aspects of attention (sustained, complex, shifting) at various times during the treatment period (p<0.05). The authors concluded that chronic supplementation with 900 mg of PSE could improve cognitive performance in a young, active population.

4.8.2. Mentha spicata L. Non-clinical Safety

The toxicity of the PSE has been evaluated in an oral gavage 90-day Sprague-Dawley rat study, Ames assay and mammalian chromosomal aberration assay, all conducted according to OECD and GLP guidelines and principles [148]. Dosages used in the 90-day study were 0, 422, 844, and 1948 mg/kg bw/day, which corresponded to equivalent dosages of rosmarinic acid of 0, 65, 130, and 300 mg/kg bw/day, respectively. PSE (and corresponding rosmarinic acid) treatment for 90 days in rats did not result in any significant effects on body weight, feed consumption, neurological parameters (assessed using a functional observational battery), hematology, clinical chemistry, gross pathology, or histopathology. There were statistically significant effects in absolute and relative weights of the pituitary gland, thyroid gland, and salivary gland in mid- and/or high dose groups in

either males or females; however, there were no corresponding microscopic changes. Thus, the noted effects were considered non-adverse, and the NOAEL for the study was 1948 mg/kg bw/day.

The PSE extract was negative for mutagenicity in the Ames assay (using TA98, TA100, TA 102, TA1535, and TA1537). There was no evidence of clastogenicity, based on the chromosomal aberration assay in human peripheral blood lymphocytes.

Although studies have not been conducted to assess the effects of PSE on developmental and/or reproductive endpoints, it is promising that no changes were observed in testes or ovaries from the above-described 90-day rat study. Until further work is conducted, cautionary labeling against use in pregnancy or lactation may be warranted.

5. SUMMARY

For the first time in US history, the older population will outnumber children by the year 2030, according to the most recent Census Bureau data on national population projections [5]. Further projections indicate that by 2034 there will be 77 million people 65+ years of age in the US. A similar trend is occurring in other regions of the world [149]. Global consumer marketing trends also indicate that today's aging population may not conform to previous demographic expectations. Most mature consumers want to stay independent for as long as possible; and they are seeking products to help them achieve physical and mental health.

In parallel to the increasing trend in the aging population, the trajectory of the US dietary supplement market continues to expand with total sales topping $9.6 billion in 2019 [150]. Thus, it is not surprising that manufacturers of dietary supplements are investing in research and development of products to meet the needs of this niche market. We chose to focus our review on eight botanicals that have a history of traditional and current marketed use for nootropic endpoints including cognition, focus, memory, and mood. Additionally, botanical-based brain health dietary supplements are seeing significant market growth as consumer's confidence in the quality of dietary supplements continues to increase [2].

The most robust clinical evaluation of safety, tolerability, and efficacy, and non-clinical assessment of toxicity of the botanicals reviewed herein typically occurs when patented and proprietary, standardized extracts are available. In general, the botanicals reviewed had some improvement in at least one efficacy outcome measured across clinical studies. The least positive clinical efficacy data seemed to be for Gotu kola, which did not show a benefit for cognitive function [97]. Results from these same studies with Gotu kola did indicate some improvement in working memory and mood; however, the results were self-reported. A more recently developed standardized extract of Gotu kola, ECa 233, has been tested in human safety, tolerability, and pharmacokinetic studies [99]. Human plasma data for ECa 233 extract indicates it is well-tolerated and that at least the metabolites of the primary constituents reach the systemic circulation. Perhaps future clinical efficacy studies with this standardized extract to address cognitive function outcomes will prove more promising.

The three botanicals that are members of the family Laminaceae; lemon balm, sage, and spearmint all showed positive results for the various clinical outcomes studied. For example, we reviewed five robust clinical studies, all of which showed positive outcomes on sleep and anxiety (mood), and certain aspects of working memory. Two independent reviews prior to ours assessed the efficacy of sage for cognitive function outcomes [125, 126]. Both reviews concluded that there was evidence to suggest that sage could improve cognitive function and alertness. Lastly, a proprietary standardized extract of dried spearmint leaves that contains 15.4% rosmarinic acid has shown positive results in at least 3 clinical studies to assess potential nootropic effects in both elderly subjects with some cognitive impairment and young healthy populations.

A predominant phytochemical constituent of Lamiaceae species is rosmarinic acid. Rosmarinic acid has been shown to inhibit acetylcholinesterase (AChE) inhibitory activity in *in vitro* experiments with the isolated enzyme [143]. This may in part provide a pharmacological explanation for positive findings on cognitive endpoints for botanicals like lemon balm, sage, spearmint, and others we did not review such as rosemary, thyme and peppermint. Lamiaceae species, and other botanical families reviewed above contain numerous other polyphenols like rosmarinic acid that can also have antioxidant properties that may play a role in cellular protection.

The botanicals included in this review were generally well-tolerated with little or no adverse events reported across studies. This finding was consistent whether studies were conducted as a single acute dose or taken chronically. Clinical data, or even traditional use, may be lacking in children, so this should also be kept in mind when considering use in this sensitive population.

From a toxicity standpoint, many of the botanicals have a significant history of safe human use in the diet (*e.g.*, lemon balm, sage, spearmint). Other botanicals like American ginseng may have fewer non-clinical studies available, but the safety for American ginseng (*Panax quinquefolia*) can be bridged using data from the more well-studied Asian ginseng (*Panax ginseng*). This should only be done when phytochemical constituent characterization data is available for species under comparison or even between standardized extracts of the same species. Chronic animal toxicity studies are also often lacking; however, this may be covered by traditional or significant history of use, and/or chronic studies conducted in human clinical trials.

The most common data gap across botanicals was the lack of information related to developmental and reproductive effects. For a botanical like Holy basil, it is fairly well established that it should be avoided during pregnancy; and contraindicated through labeling. Data for the safe use of American ginseng during pregnancy is equivocal based on findings from *in vitro* and *in vivo* studies. This highlights another critical outage in available toxicity data for most botanicals. Even when toxicity studies are conducted, they

are often not done so according to international harmonized guidelines. Thus, findings are often difficult to interpret.

A consideration should also be given to potential botanical-drug interactions, especially considering that the targeted consumer for brain health dietary supplements may be the elderly. The elderly population, particularly in the West, has a significant polypharmacy use. Clinical botanical-drug interaction studies have confirmed a lack of effect for *Ginkgo biloba* and ginseng spp. [151]. The other botanicals have either not been studied or only have *in vitro* data available.

Assessing clinical studies with nootropic outcomes can be difficult due to the numerous types of tests that are used, making comparisons across studies challenging. Often, differences in the types of tests used vary based on geographical regions in which the studies were conducted. Comparing data for accomplishments of various outcome tasks between healthy individuals and diseased populations (*e.g.*, Alzheimer's) may also show very different results. In numerous examples from the studies reviewed above, a botanical may show promising effects on attention or focus in young healthy individuals; but have no significant effect in older populations with some cognitive impairment. Also, studying one task may affect another part of cognitive function (*e.g.*, testing attention may affect working memory).

If studying nootropic endpoints is not difficult enough, the addition of botanical extracts as the testing agent adds even more complexity. Especially in older clinical (and non-clinical) studies, botanical extracts were not well-characterized, and/or standardized extracts were not used. This situation makes it nearly impossible to compare data across studies. Fortunately, the availability of standardized extracts is addressing this deficit. Future clinical work on the beneficial properties of standardized botanical extracts on nootropic endpoints would be aided by larger trials using consistent testing methods.

There are other well-known botanicals currently used as dietary supplements that are only now emerging as potential nootropics. For example, an *Echinacea angustifolia* root extract has just recently been tested in a randomized, double-blind, placebo-controlled trial in subjects with anxiety [152]. Findings from this study were promising and showed a significant anxiolytic effect. Additionally, the role of combination botanical products on cognitive endpoints could also significantly improve efficacy. Since the structure and function of the brain are dependent on a number of other nutrients that have also been studied for cognitive function properties (*e.g.*, fish oil, omega-3, amino acids, vitamins, *etc*.), botanical-nutrient-vitamin-mineral combinations should also be studied.

Of the eight botanicals studied for various nootropic activities, all show clinically meaningful, with the exception of Gotu kola. Gotu kola did not show efficacy for various cognitive function endpoints; however, it may improve endpoints associated with mood and sleep. It should be noted that Gotu kola has not been as well studied as the other botanicals reviewed, so further study might provide different conclusions. The most robust clinical data exists for the well-characterized and standardized extracts available for botani-

cals such as *Bacopa monnieri, Ginkgo biloba*, and *Mentha spicata*. The botanicals Holy basil, American ginseng, and *Salvia* spp. are also less well-studied but do show positive findings for cognitive endpoints. Lemon balm has been more studied for effects on mood, stress relief, and sleep; and results are also positive. The clinical safety profile of these botanicals indicates that they are well-tolerated. From a non-clinical safety perspective, data gaps, particularly related to developmental and reproductive endpoints and in many cases chronic use for these botanicals may need further attention.

LIST OF ABBREVIATIONS

AARP	=	American Association of Retired Persons
AVLT	=	Auditory Verbal Learning Test
B-L VAS	=	Bond-Lader Visual Analogous Scale
BW	=	Body Weight
CARKO	=	Constitutive Androstane Receptor Knockout
CDR	=	Cognitive Drug Research Battery
CVLT	=	California Verbal Learning Test
EFSA	=	European Food Safety Authority
EMA	=	European Medicines Agency
ESCOP	=	European Scientific Cooperative on Phytotherapy
FDA	=	Food and Drug Administration
HADS	=	Hospital Anxiety Depression Scale
INDCA	=	*Centella asiatica* (CA) L. standardized extract
mHAM	=	Modified Hamilton Anxiety Scale
MMSE	=	Mini-Mental State Exam
MTF	=	Multi-Task Framework
NO(A)EL	=	No Observed (Adverse) Effect Level
NTP	=	National Toxicology Program
OECD	=	Organization for Economic Co-operation and Development
PAMDI	=	Possible Average Maximum Daily Intake
POMS	=	Profile of Mood States
PSE	=	Proprietary Spearmint Extract
PSS	=	Perceived Stress Scale
RVIP	=	Rapid Visual Information-Processing Task
US	=	United States
WAIS-IV	=	Weschler Adult Intelligence Scale

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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