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Effects of *Acacia catechu* and *Scutellaria baicalensis* extract on cognitive function in a healthy adult population: a double-blind, randomized, placebo-controlled trial

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ABSTRACT
Flavonoids, known for their neuroprotective properties, are abundant in *Acacia catechu* and *Scutellaria baicalensis*. Yet, human studies on their combined effects are limited.
Objective: This study evaluated the cognitive effects of combined *Acacia catechu* and *Scutellaria baicalensis* supplementation in healthy adults.
Methods: In a randomized, double-blind, placebo-controlled trial, 26 males and 59 females ($N = 85$; 43 ± 8 yrs) consumed the test product (TP) containing 240 mg stem extract of *Scutellaria baicalensis* and 51 mg heartwood extract of *Acacia catechu* (UP326, Unigen, Tacoma, WA USA) or placebo (PLA) for four weeks. Cognitive function and biomarkers were assessed throughout the study.
Results: Significant time effects ($p < 0.001$) were observed across cognitive function assessments, with no differences between groups. Energy and fatigue reports showed a significant time effect ($p = 0.023$), while no significant differences emerged in general health and well-being scores. Cortisol levels increased significantly over time across conditions ($p = 0.005$), but no significant changes were observed in change scores or individual visits. Interim ($p = 0.023$) and final ($p = 0.004$) absolute basophil levels differed significantly between groups, with no intergroup changes. No significant differences in BDNF, CRP, or health and safety biomarkers were detected between supplemental conditions or over time.
Discussion: Four-week daily TP supplementation significantly enhanced cognitive function without difference from placebo. However, no adverse events or significant blood marker changes were noted, suggesting TP supplementation is generally well-tolerated. Further research is warranted to explore the preventive and attenuating cognitive effects of this supplementation.

Trial registration: ISRCTN.org identifier: ISRCTN16548309.

KEYWORDS
Flavonoids; nutraceuticals; cognitive function; inflammation; neuroprotection; herbal supplements; Acacia catechu; Scutellaria baicalensis; biomarkers

Introduction

Due to a myriad of factors including physiological, emotional, and societal considerations, cognitive function and information processing can be impacted as individuals progress through life. Extending beyond overt declines in cognitive potential, challenges to attention, focus, and concentration can all impact cognitive performance. Previous studies have documented oxidative stress related declines in hippocampal information processing, which can be reflected in deficits in spatial learning and memory formation, as well as a reduction in long-term potentiation, which is essential for memory consolidation [1–3]. Consequently, there is increasing interest in early interventions to mitigate the typical declines in cognitive performance.

Across centuries, medicinal plants have gained recognition for their ability to influence different aspects of health, including cognitive well-being. Polyphenol-rich dietary supplements are of interest for potential neuroprotective benefits, guarding against oxidative and inflammatory insults, which can ultimately enhance neuronal function. Notably, flavonoids, a specific subclass of polyphenols, have been the subject

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of extensive research for their promising impact on numerous health-related factors including antioxidant, anti-inflammatory, and anti-apoptotic properties [4]. Nutritional extracts derived from *Scutellaria baicalensis* (*S. baicalensis*) and *Acacia catechu* (*A. catechu*), which are rich sources of flavonoids, have been investigated for their potential therapeutic benefits both separately and in combination. *S. baicalensis* is widely distributed in North China, Japan, Korea, Mongolia, and Russia while the roots are used in Huang-Qin, one of the most popular traditional Chinese medicinal practices [5,6]. The extracts and major chemical constituents of *S. baicalensis* have been reported to exhibit a range of beneficial properties including anti-viral, anti-tumor, anti-bacterial, antioxidant, anti-inflammatory, hepatoprotective, and neuroprotective activities [7]. Similarly, *A. catechu* has widespread use across China, Taiwan, and Japan for its health properties (Stohs and Bagchi [8]). The flavonoids found in *A. catechu* extracts and related polyphenols have been shown to possess antioxidant, anti-inflammatory, and anti-apoptotic effects with many flavonoids demonstrating neuroprotection against neural injuries and neurodegenerative disease [9].

Most recent research surrounding the combined and individual use of *S. baicalensis* and *A. catechu* as nutritional supplements has been largely limited to animal models. For instance, *S. baicalensis* flavonoids have been found to improve spatial learning and memory in rats with vascular dementia [10], as well as attenuate oxidative stress-induced apoptosis in Sprague–Dawley cortical neurons exposed to cyanide [11]. Similarly, catechin from *A. catechu* has demonstrated anti-amnesic and antioxidant effects in mice with cognitive deficits [12]. When considering the safety of these herbal supplements in combination, a proprietary extract combination of *S. baicalensis* and *A. catechu,* UP446*,* for 1–12 weeks in animals did not result in any adverse effects [13–15]. Limited human research has also reported positive safety outcomes on A. *catechu* and *S. baicalensis*. For example, Morgan et al. [16] demonstrated a high degree of safety with a 250 mg dose taken twice daily for 12 weeks in 59 healthy men and women. Additionally, the safety of flavocoxid, a proprietary combination of these two herbal extracts, has been demonstrated in a 4-week human study in 103 healthy men and women involving a 500 mg twice daily dose [17].

Given the potential benefits of a supplement with anti-inflammatory, antioxidative, and neuroprotective properties, such a supplement may also be useful for addressing memory impairment associated with inflammation and oxidative stress. Yimam et al. [18] demonstrated that aged rats consuming a proprietary blend, UP326, of a standardized baicalin extract from *Scutellaria* and catechin extract from *Acacia* at doses of 7 and 34 mg/kg (70 kg male human equivalency: 79 and 384 mg/day, respectively) for 11 weeks exhibited fewer errors in a radial arm water maze compared to those consuming a lower dose (3 mg/kg, or 34 mg/day human equivalency) or those that consumed nothing, indicating improved short-term memory. However, there remains limited human research on the mechanism and effectiveness of the combination of *S. baicalensis* and *A. catechu* on cognitive function, processing speed and accuracy, and systemic inflammation. Thus, the purpose of this study was to examine the impact of a four-week supplementation regimen of *S. baicalensis* and *A. catechu* on cognitive function in healthy, middle aged males and females.

Methods

Experimental design

The study was conducted using a randomized, double-blind, placebo-controlled design, in accordance with the Declaration of Helsinki. Lindenwood University Institutional Review Board approved the protocol (IRB-22-78, approval date: February 17, 2022) and participants provided written informed consent prior to their participation. Using G*Power 3.1 [19], an a priori power analysis was performed to determine that 100 participants would provide an estimated power ($1 - \beta$) of 0.90 to detect a difference of 0.656 times the standard deviation between groups, using an alpha (α) level of 0.05. Eligibility was determined through a health history screening. Four in-person visits included screening (Visit 1), baseline testing (Visit 2, Day 0), interim (Visit 3, Day 15 ± 3), and end of study (Visit 4, Day 29 ± 3). Participants consumed identical looking capsules twice per day of a placebo (PLA) or the test product (TP) for 29 ± 3 days. The trial was retrospectively registered at ISRCTN.com (ISRCTN16548309) on 11 November 2023.

Material

The PLA capsule contained 250 mg of Maltodextrin. Each TP capsule contained a combination of active ingredients: 240 mg standardized bioflavonoid extract (with not less than 70% Baicalin) derived from the stems of *S. baicalensis*, 51 mg standardized bioflavonoid extract (with not less than 90% catechins) derived from the heartwood of *A. catechu*, and 9 mg maltodextrin (UP326 Unigen, Tacoma, WA USA). Detailed methods for the preparation and quantification of the two major flavonoids, baicalin and catechin from *S. baicalensis* and *A. catechu*, respectively, were disclosed in a US patent [20].

Participants were assigned to the PLA and TP groups using a double-blind randomization process with a 1:1 allocation ratio. A third-party affiliate, with no direct involvement in any study procedures, performed the concealment of all study products. The third party used a 1:1 allocation randomization scheme provided by the study sponsor to assign a unique randomization number to each supplement container. To protect concealment, the capsules and containers were identical in appearance. The concealment process ensured that all members of the research team and all participants remained blinded to the group allocation throughout the study. The research team was only un-blinded following the finalization of the analysis.

Study participants

Healthy adults (26 male, 59 female) aged 30–60 years, with a body mass index (BMI) 18.0–34.9 kg/m^2 were randomized. Baseline demographics are detailed in Table 1. Eligible participants agreed to refrain from: antidiabetic, blood sugar lowering, or blood-thinning medications, supplements with a known impact on cognition, changing their diet or lifestyle significantly throughout the study. Those with childbearing potential agreed to use appropriate birth control. Participants were excluded if they: were lactating, pregnant, planning to become pregnant, had known sensitivity or allergy to study products, had undergone major surgery in three months prior to or planned on having major surgery during the study protocol, had a pacemaker or implantable cardiac defibrillator, an abnormality or obstruction of the gastrointestinal tract precluding swallowing or digestion, substance abuse history, recent COVID-19 vaccination, current COVID-19 infection, or post-COVID-19 condition as per WHO definition. Further, participants with diagnosed diabetes, heart disease, uncontrolled hypertension, renal/hepatic disorders, unstable thyroid disorders, immune disorders, affective/neurological/psychiatric disorders, cancer within five years, and any condition posing risk or influencing results were excluded. Receipt or use of test products in another research study within 28 days prior or any other active/unstable medical conditions, or medications/supplements/therapies posing a risk to the participant or study outcome were reasons for exclusion.

Table 1. Subject demographics.

All Participants (n = 85)	TP Mean (SD)	PLA Mean (SD)
Age (years)	44 (8)	43 (8)
Weight (kg)	77.7 (16.6)	78.2 (15.8)
Body Mass Index (kg/m^2)	27.2 (3.7)	26.8 (3.8)
Fat Free Mass (kg)	30.0 (8.0)	30.9 (8.4)
Bodyfat (%)	30.6 (8.4)	29.5 (8.1)
Resting Heart Rate (beats/min)	68 (10)	66 (11)
Systolic Blood Pressure (mm Hg)	120 (13)	117 (13)
Diastolic Blood Pressure (mm Hg)	80 (8)	78 (8)

Data are presented as means (SD); kg = kilograms; m = meters; PLA = placebo; TP = *Scutellaria baicalensis* and *Acacia catechu*.

Procedures

Anthropometric and hemodynamic assessments

Height was measured to the nearest ± 0.5 cm using a wall-mounted stadiometer (HR-200, Tanita Corp, Inc. Tokyo, Japan) during screening. Body mass was measured to the nearest ± 0.1 kg at each visit using a self-calibrating digital balance (Tanita BWB-627A, Tokyo, Japan). Participants with a body mass change exceeding 2% between visits were excluded for non-weight stability. Resting heart rate and blood pressure, taken after five minutes of quiet, seated rest, were measured at each visit using an automated sphygmomanometer (Omron BP785, Omron Corporation, Kyoto, Japan).

Body composition analysis

Fat-mass and fat-free mass (FFM) were assessed at baseline using a bioelectrical impedance analyzer (InBody 570, InBody, South Korea). Participants removed all items from their pockets, jewelry, and heavy clothing. They enhanced electrical conductivity by wiping hands and feet with an InBody wipe. The analyzer, calibrated daily according to manufacturer recommendations, required participants to have both hands and feet in contact with the sensors.

Venous blood collection and processing

During visits 2, 3 and 4, venous blood was collected via a forearm vein using standard venipuncture. Blood samples were collected into EDTA-coated Vacutainer™ tubes and serum separation tubes, inverted ten times, and centrifuged at 3000 rpm at 4°C for 20 min (Mega-Fuge R8, Thermo Fisher Scientific, Waltham, MA, USA). Serum separation tubes were clotted for one hour before centrifugation. Plasma and serum were frozen at −80°C within two hours for later analysis.

Inflammation, brain health, and oxidative stress blood markers

During visits 2, 3, and 4, duplicate measurements of plasma C-reactive protein (CRP), cortisol, and brain-derived neurotrophic factor (BDNF) were performed using quantitative enzyme-linked immunosorbent assay techniques (CRP: EIA-3954, cortisol: EIA-1887R, BDNF: EIA-5968R, DRG International, Springfield, NJ, USA) following the manufacturer's instructions. Serum uric acid concentrations were analyzed at a commercial diagnostic laboratory (Quest Diagnostics).

Blood chemistry and hematology

For safety assessment following supplementation, whole blood and serum samples were analyzed at a commercial diagnostic laboratory (Quest Diagnostics). Whole blood samples were assayed in plasma for complete blood counts, including red/white blood cell count, platelet count, hemoglobin, hematocrit, red blood cell dimension width (RDW), mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH), mean corpuscle hemoglobin content (MCHC), and % and cell count of neutrophils, lymphocytes, monocytes, eosinophils, and basophils % and cell count (granulocytes -> neutrophils, eosinophils, basophils) to evaluate the safety of supplement ingestion. Serum samples underwent a comprehensive metabolic panel (CMP), including albumin, albumin/globulin ratio (calculated), alanine aminotransferase (ALT), aspartate aminotransferase (AST), BUN/creatinine ratio (calculated), calcium, carbon dioxide, chloride, creatinine with estimated glomerular filtration rate (eGFR), globulin, glucose, potassium, sodium, total bilirubin, total protein, and urea nitrogen.

Cognitive testing battery

Cognitive assessments were completed on an electronic tablet (3rd Generation; Apple Inc., Cupertino, CA, USA) during visits 2, 3, and 4 using the iPad version of the NIH Toolbox, a cognitive battery that normalizes scores for age, sex, education, and ethnicity. The tests included the Flanker Inhibitory Control and Attention (Flanker), the Dimensional Change Card Sort (DCCS), and the Pattern Comparison Processing Speed (PCPS) [21]. Participants received oral and visual instructions, made selections with their dominant hand, and were scored for accuracy and reaction time using a scoring algorithm. All reaction times for correct items were reported in seconds from a standardized home base position to making the item response. Scores reflected performance relative to a nationally representative normative sample.

To assess cognitive interference and visual attention, an electronic Stroop color-word task (Stroop) was performed in a seated position with minimal distractions during visits 2, 3, and 4 using an electronic tablet application (Brain Test – Stroop Effect, Attila Hegedus, v1.5.0). The Stroop task was performed as previously explained by Scarpina and Tagini [22]. The participants completed three 60-second trials, and the best score was recorded. The Trail Making Test, parts A (TMT-A) and B (TMT-B), followed methodology previously published [23] during visits 2, 3, and 4 to assess attention, flexibility, and visual search and scanning [24].

Health related quality of life

Health related quality of life was measured at visits 2, 3, and 4 using the RAND 36-Item Short Form Health Survey (SF-36) (RAND, Santa Monica, California, USA) covering eight health concepts: physical functioning, pain, role limitations (physical and emotional), emotional well-being, social functioning, energy/fatigue, and general health.

Supplementation protocol

Participants received random assignments of either PLA (microcrystalline cellulose) or TP (UP 326, Unigen, Tacoma, WA USA) for 29 ± 3 days. Participants took two daily doses (every morning and evening) with food and water. If a dose was missed, it could be taken later that day, but not made up if it extended into the next day. The initial dose was given in the lab during visit 2, and participants self-administered at home thereafter. Compliance, assessed at each visit with a paper diary, was deemed compliant between 80% to 120%.

Adverse events

Adverse Events (AEs) were systematically collected through spontaneous participant reporting, clinical evaluation, team interactions, and file reviews. All recorded events were systematically categorized using MedDRA system organ class and graded using Common Terminology Criteria for Adverse Events ([CTCAE] Version 5.0, U.S. Department of Health, and Human Services (published: 27 November 2017)). Data on frequency, severity, duration, and outcome of AEs deemed related or potentially related to study involvement were securely recorded in an electronic database.

Statistics

Statistical analysis was performed using JASP Team (Version 0.14.1). The data were checked for normality and ensured that the assumptions of the respective statistical tests were met. If the assumption of normality was violated, the data was transformed and reassessed for normality. Nonparametric methods were implemented if normality persisted and noted in the results. The threshold for statistical significance was set at $p \leq 0.05$, and the data are presented as mean ± standard deviation (SD).

To examine changes over time (baseline, interim, final) between conditions (PLA and TP) for normally distributed dependent variables, a mixed factorial ANOVA was employed. In case of violation of the assumption of heteroscedasticity for repeated measures, a Greenhouse–Geisser correction factor was applied. If a significant interaction (condition x time) was found, simple main effects analysis was conducted with repeated measures ANOVA within each condition. For significant ANOVAs, partial Eta squared (η_p^2) effect sizes, where 0.01, 0.06, and 0.14 were considered small, moderate, and large effects, respectively, were reported. Post-hoc testing was conducted using holm correction factors. If significant differences were observed between conditions, the magnitude of the effect using Cohen d effect size, with 0.2, 0.5, and 0.8 considered small, moderate, and large effects, respectively, was reported.

For dependent variables that were not normally distributed, change scores were calculated (Interim – Baseline and Final – Baseline) to assess changes between conditions across time. The change scores and differences between conditions at each time point were then analyzed using a Mann–Whitney U test. A Friedman test was performed to evaluate overall change across time points. If the Friedman test revealed significant results, further Friedman tests were conducted within each condition to analyze changes over time, followed by Holm-adjusted Conover post hoc tests.

Results

Participant flow

The Consolidated Standards of Reporting Trials (CONSORT) diagram for this study is presented in Figure 1. Out of 322 potential participants, 122 failed to schedule a first visit, 92 failed pre-screening, and the remaining 108 were consented. Of these, one

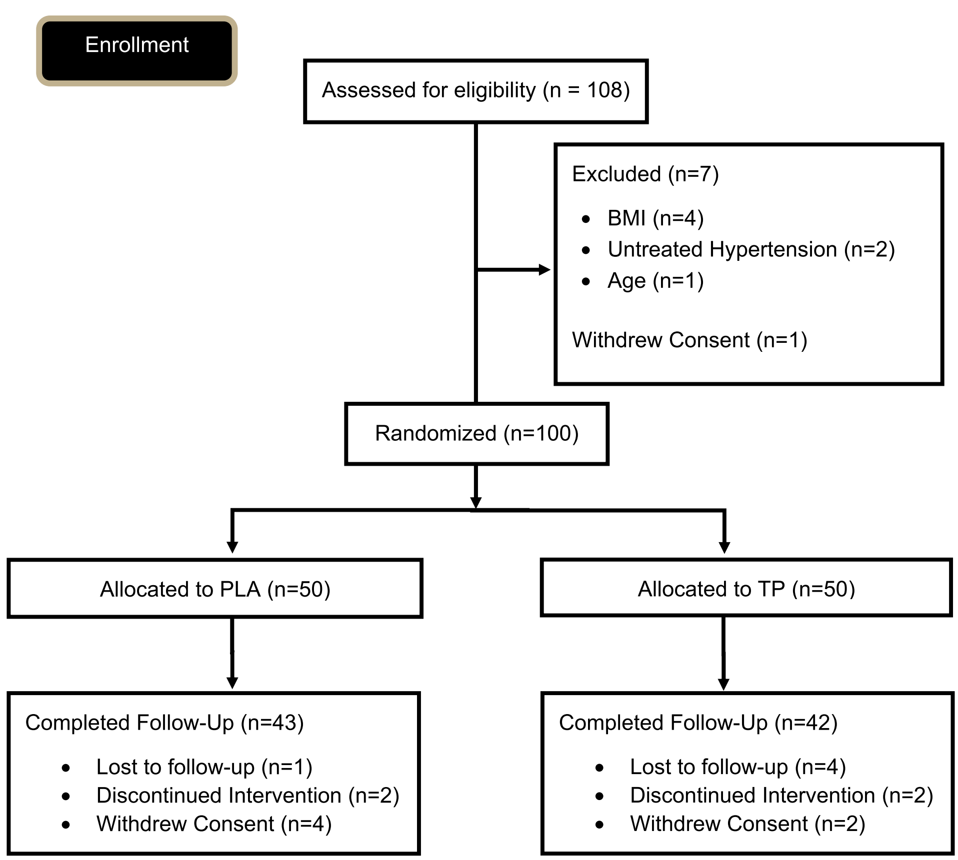

Figure 1. CONSORT diagram.

declined to participate and seven did not qualify. Of the 100 qualified participants, 85 successfully completed the intervention (PLA: $n = 43$, TP: $n = 42$) due to various circumstances including participant withdrawals, loss to follow-up, and discontinuation. This resulted in the study being slightly underpowered compared to the initial calculation.

Body composition & hemodynamics

We observed no significant ($p > 0.05$) condition x time interaction, main effect of condition, or main effect of time for body mass, FFM, body fat %, BMI, systolic blood pressure, or diastolic blood. Heart rate analysis revealed no significant ($p > 0.05$) condition x time interaction. However, we did observe a significant small to moderate main effect of time in resting heart rates ($p = 0.028$, $h_p^2 = 0.043$).

Cognition tests

No baseline differences ($p > 0.05$) were observed for any of the tests included within the NIH, Stroop, or TMT measures. Analysis for NIH Flanker measures revealed no significant condition x time interaction for age-corrected scores ($p > 0.05$) or fully corrected T-scored ($p > 0.05$). However, large significant main effects of time were observed for Flanker age-corrected scores ($p < 0.001$, $\eta p^2 = 0.389$), and fully corrected T-scores ($p < 0.001$, $\eta p^2 = 0.378$).

NIH DCCS measures revealed no significant condition x time interaction for age-corrected scores ($p > 0.05$) or fully corrected T-scores ($p > 0.05$). However, moderate significant main effects of time were observed for DCCS age-corrected scores ($p < 0.001$, $\eta p^2 = 0.122$), and fully corrected T-scores ($p < 0.001$, $\eta p^2 = 0.132$).

NIH PCPS measures were not normally distributed and analyzed using non-parametric statistics. Analysis for the NIH Pattern Comparison Processing Speed revealed no significant differences in age-corrected scores ($p > 0.05$) or fully corrected T-scored ($p > 0.05$) change scores. However, a significant main effect of time was observed for age-corrected scores ($p < 0.001$), and fully corrected T-scores ($p < 0.001$).

NIH General life satisfaction revealed no significant condition x time interaction for T-scores ($p > 0.05$). However, a moderate main effect of time was observed for General life satisfaction T-scores ($p = 0.002$, $\eta p^2 = 0.074$).

Stroop assessments revealed no significant condition x time interaction ($p > 0.05$) or main effect of condition ($p > 0.05$). However, a significant large main effect of time was observed ($p < 0.001$, $\eta p^2 = 0.540$).

TMT measures were not normally distributed and analyzed using non-parametric statistics. Analysis for the TMT Part A completion time revealed no significant differences for change scores ($p > 0.05$ for both change scores). However, TMT Part A completion time revealed a significant main effect of time ($p < 0.001$).

Analysis for the TMT Part A errors revealed significant differences for change scores Final to Baseline ($p = 0.049$) with values from TP exhibiting a larger magnitude of change when compared to PL. Additionally, TMT Part-A errors revealed a significant main effect of time ($p = 0.020$). TMT Part B completion times revealed a significant main effect of time ($p < 0.001$), however no significant differences were identified for both change scores ($p > 0.05$). Analysis for the TMT Part B errors revealed no significant differences for change scores (Interim to Baseline; Final to Baseline) (both $p > 0.05$), or main effect of time ($p > 0.05$).

General health and well-Being

SF-36 general health scores revealed no significant condition x time interaction ($p > 0.05$) or main effects of condition or time ($p > 0.05$). SF-36 energy and fatigue measures revealed no significant condition x time interaction ($p > 0.05$). However, a small to moderate significant main effect of time was observed for energy and fatigue ($p = 0.023$, $\eta p^2 = 0.046$).

Multiple SF-36 variables such as emotional well-being, pain experience, physical functioning, and social functioning were not normally distributed and analyzed using non-parametric statistics. Analysis for SF-36 variables measures revealed no significant differences for change scores (both $p > 0.05$), the main effect of condition ($p > 0.05$) or time ($p > 0.05$) for emotional well-being, pain experience, physical functioning, and social functioning.

SF-36 analysis revealed significant differences between conditions at baseline for role limitations due to physical health ($p = 0.029$) and role limitations due to emotional problems ($p = 0.046$), with the PLA condition reporting lower scores compared to those using the TP. To control for these measures, we performed an ANCOVA and analyzed the interim and final time points. This analysis for both role limitations due to physical health, and role limitations due to emotional problems revealed no significant condition x time interaction ($p > 0.05$), main effect of condition ($p > 0.05$) or time ($p > 0.05$) for either variable (Table 2).

Eleven AEs deemed related or potentially related to the study products are presented in Table 3. Seven PLA participants reported eight events, including

Table 2. Cognition assessments.

	Variable	Group	Baseline Mean	Baseline (SD)	Interim Mean	Interim (SD)	Final Mean	Final (SD)	*P* value
Normally Distributed	NIH Flanker Age-Corrected Score	PLA	96.8	(13.3)	106.5	(14.9)	108.0	(15.5)	G: 0.155
		TP	92.0	(14.9)	100.9	(18.0)	104.7	(19.2)	T: < 0.001*
									G × T: 0.588
	NIH Flanker Fully Corrected T-score	PLA	46.2	(9.2)	53.3	(9.4)	54.3	(10.7)	G: 0.180
		TP	43.6	(10.0)	49.5	(12.5)	52.0	(13.1)	T: < 0.001*
									G × T: 0.610
	NIH DCCS Age-Corrected Score	PLA	113.4	(17.7)	116.3	(17.5)	118.9	(18.0)	G: 0.485
		TP	108.3	(17.4)	115.3	(18.2)	117.7	(18.2)	T: < 0.001*
									G × T: 0.340
	NIH DCCS Fully Corrected T-score	PLA	57.1	(11.8)	58.9	(11.9)	60.8	(11.9)	G: 0.494
		TP	53.2	(11.5)	58.9	(12.0)	60.0	(12.2)	T: < 0.001*
									G × T: 0.177
	NIH General Life Satisfaction T-score	PLA	50.8	(8.4)	52.0	(8.7)	52.8	(8.7)	G: 0.666
		TP	52.3	(7.7)	52.2	(6.2)	53.3	(7.5)	T: 0.002*
									G × T: 0.248
	Stroop Assessment Score	PLA	20.1	(9.6)	26.3	(12.2)	29.7	(13.1)	G: 0.610
		TP	21.5	(10.4)	27.3	(11.2)	30.8	(10.5)	T: < 0.001*
									G × T: 0.958
	Variable	Group	Baseline Median	Baseline (IQR)	Interim Median	Interim (IQR)	Final Median	Final (IQR)	*P* value
Non-Normally Distributed	NIH PCPS Fully Corrected T-score	PLA	61.0	(14.0)	71.0	(15.0)	76.0	(8.5)	I-BΔ: 0.751
		TP	57.0	(15.3)	68.5	(18.5)	74.0	(15.8)	F-BΔ: 0.939
									T: < 0.001*
	NIH PCPS Age-Corrected Score	PLA	118.0	(18.5)	132.0	(25.5)	141.0	(15.0)	I-BΔ: 0.912
		TP	112.0	(25.3)	128.0	(26.0)	137.0	(25.8)	F-BΔ: 0.979
									T: < 0.001*
	TMT Part-A Time	PLA	15.7	(5.0)	13.5	(3.8)	13.2	(3.1)	I-BΔ: 0.153
		TP	15.1	(5.0)	14.7	(4.7)	14.7	(6.2)	F-BΔ: 0.119
									T: < 0.001*
	TMT Part-A Errors	PLA	0	(0.0)	0	(0.0)	0	(0.0)	I-BΔ: 0.057
		TP	0	(0.0)	0	(0.8)	0	(1.0)	F-BΔ: 0.049
									T: 0.020*
	TMT Part-B Time	PLA	28.9	(12.1)	28.2	(11.8)	24.3	(9.1)	I-BΔ: 0.104
		TP	33.2	(19.9)	28.9	(14.6)	24.6	(12.6)	F-BΔ: 0.124
									T: < 0.001*
	TMT Part-B Errors	PLA	0	(1.0)	1	(2.0)	1	(2.5)	I-BΔ: 0.237
		TP	0	(4.0)	0	(2.0)	0	(3.0)	F-BΔ: 0.217
									T: 0.300

Normally distributed data are presented as means (SD); Non-normally distributed data are presented as median (IQR); *Significant change ($p < 0.05$); G = Main effect for group; G × T = Interaction effect; T = Main effect for time; I-BΔ = Interim-Baseline change; F-BΔ = Final-Baseline change; PLA = placebo; TP = *Scutellaria baicalensis* and *Acacia catechu; DCCS = Dimensional Change Card Sort; PCPS = Pattern Comparison Processing Speed; TMT = Trail Making Test*.

one participant with both a headache and restlessness. Among these, four AEs were mild (two headaches, one gastrointestinal issue, one restlessness), three were moderate (two gastrointestinal issues, one headache), and one was severe (gastrointestinal issue). The remaining three mild AEs were reported by two TP participants, with one participant experiencing two subsequent headaches.

Table 3. Adverse events.

Category	PLA	TP
Participants Reported	7	2
Headache	3	2
Gastrointestinal	4	0
Drowsy	0	1
Restlessness	1	0

Data are presented as counts. PLA: Placebo; TP: *Scutellaria baicalensis* and *Acacia catechu*.

Hematological parameters

No baseline differences were observed for any of the hematological CBC parameters. Absolute basophils analysis revealed a small to moderate significant condition x time interaction ($p = 0.043$, $\eta p^2 = 0.050$). Follow-up tests revealed significant differences at both the interim ($p = 0.023$, $d = 0.534$) and the final visit ($p = 0.004$, $d = 0.517$), where TP exhibited moderately higher absolute basophil counts compared to the PLA condition. Additionally, we observed a large significant main effect of condition ($p = 0.022$, $\eta p^2 = 0.082$) where TP had higher absolute basophils counts compared to PLA condition supporting our simple main effect results. However, we did not observe a significant main effect of time ($p > 0.05$), or significant changes over time within each condition ($p > 0.05$). Furthermore, no significant condition x time interactions, main effects of condition or time were observed (all $p > 0.05$) for any other CBC components (see Supplementary Data Table 1).

Comprehensive metabolic panel

CMP analysis was conducted to monitor clinical safety throughout the course of the supplementation regimen. Analysis of ALT levels revealed a small significant condition x time interaction ($p = 0.043$, $\eta p^2 = 0.043$). Despite this interaction, subsequent analyses did not demonstrate significant differences within conditions across the different visits, nor were there discernible differences between conditions at each individual visit.

AST and total bilirubin were not normally distributed and analyzed using non-parametric statistics. Analysis for both AST and total bilirubin revealed no significant differences for change scores (both $p > 0.05$), the main effect of condition ($p > 0.05$) or time ($p > 0.05$). Additionally, no significant condition x time interactions, main effects of condition or time were observed (all $p > 0.05$) for any other CMP components (see Supplementary Data Table 2).

Other biomarkers

All data for CRP, cortisol, BDNF, and uric acid were not normally distributed and analyzed using non-parametric statistics. Cortisol analysis revealed no significant differences for change scores (both $p > 0.05$). However, a significant main effect of time was observed ($p = 0.005$) for cortisol. Analysis for all other biomarkers revealed no significant differences for change scores ($p > 0.05$), the main effect of condition ($p > 0.05$) or time ($p > 0.05$) (see Supplementary Data Table 3).

Discussion

The primary objective of this study was to examine the effects of a four-week supplementation regimen with 300 mg twice per day of a combination (UP326) of two standardized bioflavonoid extracts from stems of *S. baicalensis* and heartwoods of *A. catechu* in healthy, middle-aged adults on cognitive function and biomarkers. Our findings revealed that supplementation with bioflavonoids from *S. baicalensis* and *A. catechu* was deemed overall safe and well tolerated, but ineffective at improving cognition or altering blood markers.

As individuals progress through life, physiological changes occur that are marked by alterations in immune system functionality and the emergence of chronic subclinical inflammation [25] This persistent systemic inflammatory milieu, accruing gradually over the lifespan, in conjunction with endothelial dysfunction, may precipitate heightened permeability across the blood–brain barrier, thereby instigating microglial activation and the initiation of neuroinflammation [26]. Consequently, these processes may culminate in compromised neuronal integrity, cerebrovascular function, and cognitive performance, thereby manifesting as impairments in executive functions, working memory, and processing speed [27].

Recent evidence, while limited, does largely support the concept that dietary polyphenols indirectly and directly impact brain health through mechanisms of neuro- and systemic inflammation modulation, adult neurogenesis, and cerebrovascular function. Carrillo et al. [28] revealed significant improvements in executive function, including short-term memory, working memory, selective and sustained attention, and speed of processing, in healthy middle-aged adults that supplemented with a fruit- and vegetable-based extract containing polyphenols. Similarly, a human clinical pilot trial involving healthy middle-aged participants revealed that daily supplementation with 300 mg of a comparable herbal polyphenol blend for 30 days resulted in enhanced speed and accuracy in processing complex information during computer tasks compared to baseline and non-supplementing counterparts [18]. These results differ from our findings, which might be attributed to the use of different methodology of assessing cognitive function or different dosing pharmacokinetics. In our study we used the NIH toolbox which has been well-validated and is a widely used assessment tool for cognitive function whereas other assessment methods were utilized in the aforementioned studies. Given that we did report an increase over time in both conditions this would indicate a potential learning effect for both conditions over time. While our participants were oriented to the testing and general layout of the testing, more familiarization may have helped offset this observed change. Furthermore, biomarker analysis was conducted for BDNF, cortisol, and CRP to assess the effect of the supplement on inflammation, brain health, and oxidative stress. BDNF, a critical regulator of neuronal survival and facilitator of long-term potentiation (Bramham and Messaoudi [29]), has been connected with both short-term and long-term memory formation [30]. Previous research has linked high-flavonoid intake to increased serum BDNF levels and improved cognitive performance in older adults [31]. However, in our study, no significant differences in BDNF were observed between supplemental conditions or over time. Similarly, CRP, an inflammatory marker, showed no significant differences across time points or conditions, maintaining normal adult range values from baseline. This suggests that the healthy adult population we recruited may not have accumulated chronic inflammation to any appreciable degree that could be impacted by supplementation. Exploring this investigation in older adults or those with more pronounced baseline indicators of systemic inflammation may provide further insights. Cortisol, despite an increase over time across both conditions, had no significant differences in change scores or at any time point, and all values remained within the normal range. It is important to note that diurnal variations impact cortisol concentrations, and while participants were required to follow a 3-hour fast and maintain consistent visit times, variability in time of day for testing was not consistent for all participants. While some variability did occur, nearly all study visits were completed within a four-hour period of time. Overall, these findings contribute to our understanding of the complex interplay between herbal supplementation, cognitive function, and physiological markers.

The bioavailability and pharmacokinetics of *S. baicalensis* and *A. catechu*, key components of the oral bioflavonoid (UP326) used in the present study, are critical for interpreting our findings. *S. baicalensis* contains flavonoids such as baicalin and baicalein, while *A. catechu* contains flavonoids such as (+)-catechin and (−)-epicatechin. Moore et al. [32] demonstrated low oral bioavailability (2.2%) and a short elimination half-life for baicalin in humans, whereas baicalein showed better gastrointestinal absorption and subsequent conjugation to baicalin in the gut wall and liver in. Additionally, a rat study by Wang et al. [33] revealed significant alterations in the pharmacokinetic profiles of these bioactive components when co-administered, with reduced maximum plasma concentrations and area under the curve for (+)-catechin, (−)-epicatechin, and baicalin with baicalein remaining unaffected. An unpublished human trial of UP446, similar in composition to our UP326, demonstrated significant inter-individual variability in the baicalein pharmacokinetics, characterized by rapid absorption and a biphasic urinary excretion pattern. These complex pharmacokinetics, including potential competitive absorption and tissue-specific distribution, may explain the variability in individual responses and the limited effects on cognition observed in our study. The reduced bioavailability of certain components when co-administered could account for the less pronounced cognitive effects than anticipated.

In alignment with the safety and toxicity findings previously outlined [13–17] oral bioflavonoids (UP326) from stems of *S. baicalensis* and heartwoods of *A. catechu* supplementation in the present study did not induce any significant changes in health and safety biomarker values or result in any significant adverse events. While there was a significant difference between groups at interim and final absolute basophil levels these values were within normal clinically accepted ranges. Further, a small but significant interaction was observed between conditions over time for the liver enzymes AST and ALT. However, subsequent analysis revealed no significant differences between conditions at individual visits or in change scores for either enzyme, and mean values remained within clinically normal ranges. There was considerable variability in enzyme levels, particularly at visit 4 within the TP group, as evidenced by large standard deviations. This variability could be due to individual differences in response to the supplement or other external factors. While we implemented strict eligibility criteria to exclude individuals with underlying conditions or chronic alcohol and substance use, our study design had limitations in controlling for factors known to cause acute increases in liver enzymes in the 24–48 h before testing. This limitation may have allowed for uncontrolled factors known to cause acute increases in liver enzymes, such as alcohol consumption, intense exercise, or high-fat meals. Future studies might benefit from more stringent control measures over longer periods before testing. Despite these statistical interactions, the clinical relevance appears to be limited given the maintenance of mean values within normal ranges. Furthermore, general health and well-being, assessed with the SF-36 questionnaire, were maintained throughout the study period.

There were some limitations present in this study. First, diet was not controlled throughout the study period and baseline polyphenol consumption was not recorded. As a result, it was unknown whether each participant regularly consumed appreciable amounts of polyphenols outside of the supplement. While the time of day for each participants' study visits were recommended to be similar, this was not able to be enforced in all instances, which could have introduced some extraneous noise to our biomarker results. Further, the supplementation period was only four weeks and did not include any longitudinal follow-up. The four weeks might not suffice for measurable improvements in cognition, systemic inflammation, or brain health.

While promising outcomes have been demonstrated in prior animal research and an initial human pilot study on the combined effects of *S. baicalensis* and *A. catechu*, our current findings present inconclusive evidence regarding its effect on cognitive function in a healthy adult population. Much of the positive research on this herbal blend stems from animal models, yet the disease state and general accumulation of cognitive decline co-morbidities may be more complex than what is exhibited in diseased animal models [34]. This study's significance lies in the safety insights of this herbal blend in humans, as evidenced by the absence of adverse events and the lack of significant changes in reported adverse events and blood biomarkers. While it is reasonable to extrapolate these safety findings to an older population with established cognitive decline or disease, a comprehensive evaluation considering both safety and efficacy would be necessary in such cases. To assess the preventative nature of this herbal blend, future research should include a longer supplementation period and a longitudinal follow-up period to determine long-term cognitive functioning maintenance or decline.

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [CMK], upon reasonable request.

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