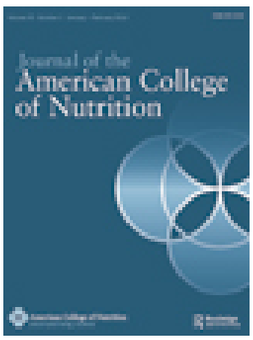

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Effect of *Alpinia galanga* on Mental Alertness and Sustained Attention With or Without Caffeine: A Randomized Placebo-Controlled Study

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ABSTRACT

Objective: Although *Alpinia galanga* has been reported to improve cognitive performance in animals, it has not been thoroughly studied for its potential psychostimulant effect in humans. A randomized, double-dummy, double-blind, placebo-controlled cross-over study was conducted to determine the effect of *A galanga* on mental alertness and sustained attention in comparison with caffeine and placebo in participants with a habitual caffeine intake.

Methods: Fifty-nine participants (18–40 years and body mass index of $\geq$18.5 and $<$25.00 kg/m^2) with moderate caffeine consumption were enrolled. The participants had a Generalized Anxiety Disorder-7 score $\leq$7, Patient Health Questionnaire-9 score $\leq$14 and a Jin Fan's Attention Network Test alertness score of 50 $\pm$ 20 ms. The interventional product (placebo, *A galanga* proprietary extract [E-AG-01], caffeine, and a combination of E-AG-01 with caffeine) was administered to the participants, followed by sequential administration of the remaining interventions on the consecutive study visits; the effects on mental alertness, sustained attention, and sleep architecture, along with safety and tolerability, were analyzed by validated methods.

Results: In the E-AG-01 group, the alertness score was increased by 11.65 $\pm$ 23.94, 12.50 $\pm$ 19.73, and 12.62 $\pm$ 0.68 ms from baseline at 1, 3 ($p = 0.042$), and 5 hours, respectively, indicating its efficacy to enhance mental alertness and the increase in alertness score as compared to placebo. In the composite group (E-AG-01 with caffeine), mean response time was significantly reduced, by 15.55 ms ($p = 0.026$) at 3 hours.

Conclusions: *A galanga* (E-AG-01) induces a beneficial effect in mental alertness and the combination of *A galanga* with caffeine impedes the caffeine crash and improves sustained attention at 3 hours. Thus, these stimulant effects might yield a new usage for *A galanga* as a key ingredient in energy drinks or similar products.

Abbreviations: AE, adverse event; ANT, Attention Network Test; BMI, body mass index; CI, class interval; E-AG-01, *Alpinia galanga* proprietary extract; GAD-7, Generalized Anxiety Disorder-7; IP, interventional product; KSS, Karolinska Sleepiness Scale; PHQ-9, Patient Health Questionnaire-9; PVT, Psychomotor Vigilance Task; SQS, Sleep Quality Scale

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Alertness; *Alpinia galanga*; attention; caffeine crash; energy drink; response time

Introduction

Caffeine is an extensively used psychostimulant. In the United States, caffeine consumption is a daily ritual for more than 80% of the population. As per recent statistics, almost 54% of Americans older than 18 drink coffee every day [1]. A survey indicated that most people consume caffeine to improve alertness, to increase wakefulness in a fatigued state, to increase performance during mentally and physically intensive situations, and to temporarily improve their memory [2, 3]. Others consume caffeine out of a habit and routine or because they like the flavor of their preferred drink [4, 5]. These effects are largely attributed to the antagonistic effect on the adenosine A_1 and A_{2A} receptors in the dopamine-rich brain areas, thus stimulating dopaminergic activity and resulting in increased wakefulness and pronounced motor activity [6–7].

Besides the psychostimulant benefits of caffeine, it has an abuse potential and may induce a psychological and physical dependence [8, 9]. Prolonged caffeine use may lead to a range of adverse effects (insomnia, palpitations, jitters, headaches, occasional lightheadedness, gastrointestinal upset, headache, chest pain, and seizures). This altered psychosomatic state, generally termed as "caffeine crash," can produce undue stress and, depending on the amount of caffeine consumed, can produce troublesome social effects in many individuals (e.g., failure to meet social obligations) [10–11]. Thus, a high dose or even a small amount of caffeine in healthy sensitive individuals can exacerbate this perceived stressful state.

In recent times, some of the untoward occurrences have drawn regulatory agencies' concern toward the uncontrolled use of caffeine in a variety of dietary supplements and energy drinks, as the main consumers of such energy products are adolescents and young adults [12]. This situation has prompted a need for an alternative ingredient that either with caffeine or as a stand-alone element provides a stimulatory benefit without the crash effect.

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Trial registration: ClinicalTrials.gov NCT02816827.
Supporting information: CONSORT Checklist (DOC), COA of Caffeine (PDF), COA of Alpinia galanga (PDF), Raw data sheet.

A large number of botanical ingredients, such as eleuthero, gingko, ginseng, rosemary, peppermint, oats, and basil, are traditionally reported to improve cognitive performance [13–14]. As evident from a vast array of scientific publications, many groups across the world have been engaged in exploring existing and newer herbal ingredients to enhance alertness and, at the same time, exhibit no adverse effect on sleep architecture [15–16]. We performed a thorough literature search on more than 50 biomasses to identify the natural substances that can improve alertness. Finally, we selected *Alpinia galanga* (Linn.) (Zingiberaceae) and two other herbal extracts on which a preliminary screening study was conducted to analyze their central nervous system (CNS) stimulant potential to confirm the literature reports. The results indicated a remarkable psychostimulant potential of *A galanga* (research code: E-AG-01) [17].

A galanga is generally used as a culinary spice [18]. Also, its traditional use is well documented for the treatment of various diseases due to its anti-inflammatory, analgesic, hypoglycemic, anti-allergic, antimicrobial, gastroprotective, antioxidant, antiplatelet, anticancer, immune-stimulatory, and cholesterol-reducing effects [19].

Although *A galanga* has been reported to improve cognitive performance in animals [20], it has not been thoroughly studied for its potential psychostimulant effect in humans. The principal objective of this study was to develop a safe alternative psychostimulant for increasing mental alertness without disturbing sleep architecture. In the current study, we exclusively attempted to analyze the efficacy of *A galanga* on mental alertness and sleep architecture of healthy young volunteers by a profound assessment of the specific parameters related to the important aspects of the attention network using standardized tests and questionnaires.

Materials and methods

Participants

The study was reviewed by an independent ethics committee (Aditya Ethics Committee, Ahmedabad, India; registered with the Office for Human Research Protections, U.S. Department of Health and Human Services, #IRB00006475) and approved on February 9, 2016. This clinical trial was registered in the trial registry before enrollment of the participants (ClinicalTrials.gov ID #NCT02816827). The study was initiated on March 2, 2016, and the last participant's last visit was conducted on August 24, 2016. Potential volunteers were contacted through multiple sources including an in-house healthy volunteers' database and a consumer group's survey agency. All participants were screened for demographic parameters (age, gender, height, weight, and body mass index [BMI]) at the screening visit to confirm compliance with the protocol. Male and female participants who were 18 to 40 years old, healthy, nonsmoking, alcohol-abstaining, and caffeine-habituated with minimal computer literacy were enrolled in the study. Caffeine history was recorded to ensure that the participants were acquainted with caffeine's stimulant effect and were not caffeine-sensitive. Participants with a BMI 18.50 to 25.00 kg/m^2, resting blood pressure $\leq$140/90 mm Hg, and an alertness score (Jin Fan's Attention Network Test [ANT], version 1.3.0) of 50 $\pm$ 20 ms at the screening visit and subsequent study visits were considered eligible. Participants with a Generalized Anxiety Disorder Screening-7 (GAD-7) score $\geq$7 and a Patient Health Questionnaire-9 (PHQ-9) score $\geq$14 were excluded, as a higher score is associated with mood disturbances and clinical conditions that are known to impact the endpoints of the study [21, 22]. Pregnant or breastfeeding women were excluded, and those currently in their menstrual period were included only after the last day of menstrual flow. Women consuming oral contraceptives were included in the study only after switching to barrier contraceptive use and a washout period of 7 days from the last dose of the oral contraceptive. Any concomitant therapy was strictly prohibited during the course of the study. All participants were provided with a demonstration of the study procedures and relevant instructions. An informed consent form to participate in the study and authorization for release of relevant protected health information to the study investigator were obtained from each of the enrolled participants.

Study design

This was a single center, randomized, double-dummy, double-blind, placebo-controlled crossover study that was designed, conducted, analyzed, and reported in accordance with ethical guidelines (Declaration of Helsinki, International Conference on Harmonization Good Clinical Practices) to protect the safety, well-being, and confidentiality of the participants and also to maintain the authenticity and credibility of the data. The study was monitored and audited by Vedic Lifesciences' clinical research team to comply with protocol and applicable regulatory guidelines.

Interventions

Participants were divided into four treatment arms and randomized (based on the randomization chart, SPSS version 20.0, IBM India) for allocation to one of the interventional products (IPs) on each study visit, and a similar trend was followed for the subsequent visits. The remaining interventions in consecutive visits were only administered after a sufficient washout period of not less than 5 half-life of caffeine in the blood to avoid a carryover effect. The IPs included placebo, *A galanga* proprietary extract (E-AG-01), caffeine, and a combination of E-AG-01 with caffeine (composite). All treatments were administered to the participants in the form of capsules, which were identical in appearance and were packed in duly labeled high-density polyethylene bottles. The blinding codes were secured at the site in the tamper-evident sealed envelopes with no access to the study team. Thus, the double-blind nature of the study was ensured and strictly followed. The participants receiving only *A galanga*

Table 1. Composition of treatments.

Ingredients	Placebo (mg/Capsule)	E-AG-01 (mg/Capsule)	Caffeine (mg/Capsule)
Alpinia galanga rhizome extract	—	300	—
Caffeine (anhydrous)	—	—	200
Microcrystalline cellulose	550	250	350
Total	550	550	550

E-AG-01 = *Alpinia galanga* proprietary extract.

or caffeine or placebo were co-assigned to an additional placebo capsule to achieve a double-dummy design identical to the caffeine + E-AG-01 regime. The composition details of all IPs are listed in Table 1. E-AG-01 (commercially available as EnXtra®) was derived from the taxonomically authenticated (by DNA barcoding) galanga rhizomes. It was a water-soluble, methyl eugenol–free extract and standardized for polyphenol-, polysaccharide-, and pyrocatecollic-type tannins. Pure anhydrous caffeine (99.7%) was supplied by Shri Ahimsa Mines and Minerals Ltd. (Jaipur, India).

Test visit procedure

Participants reported to the clinic during morning hours and the testing began at an early time of the day (8AM–9AM) for each visit to avoid influence of daily challenges (related to mental and physical stress) on the study outcomes. Also, the time of the day was matched for all four visits to reduce variability in response due to diurnal patterns. The participants reported to the clinic following 24-hour abstinence from caffeine-containing products or any psychostimulants prior to all study visits. The participants were also instructed to obtain sufficient sleep during the night prior to testing, which was confirmed by an updated sleep diary. Upon arrival at the clinic, the participants were asked to relax for 15 to 20 minutes, after which vital parameters were measured and a standardized meal of approximately 200 calories was provided to control variations from possible dietary confound. The baseline data were collected at 30 minutes postbreakfast followed by the administration of an IP, wherein one dose of the product was administered to the participant by a trial coordinator at the investigational site.

The study tools were administered at 1, 3, and 5 hours post–IP administration and the data were collected. No other food or calorie-containing beverages were provided during this period. The participants were allowed to drink water as desired and to relax in an isolated room at a comfortable temperature with free access to a computer or magazines during the clinic stay period. Any psychostimulating activity was strictly prohibited during this period. To analyze the effect of the IP on sleep architecture, a second dose of the corresponding IP was supplied in a labeled bottle to be taken before dinner the same night of the clinic visit, and the participants were asked to record all details pertaining to sleep quality and duration of sleep in the sleep diary. To ensure treatment compliance, product accountability was monitored by a clinical research coordinator on the following visit.

Screening tools

Due to the lack of literature pertaining to the effect of *A galanga* in mentally unstable or depressed individuals, we decided to conduct the study in mentally stable population to limit confounding factors and ambiguous results from such a study population. With the aid of the screening tools GAD-7 and PHQ-9, we enrolled only participants with stable qualifying scores.

GAD-7 is a valid and efficient screening tool for generalized anxiety disorder and assessing its severity in clinical practice and research. It is moderately good at screening three other common psychological conditions: panic disorder (sensitivity = 74%, specificity = 81%), social anxiety disorder (sensitivity = 72%, specificity = 80%), and posttraumatic stress disorder (sensitivity = 66%, specificity = 81%)[23, 24].

PHQ-9 is a multipurpose tool for screening, diagnosing, monitoring, and measuring the severity of depression. It rates the frequency of symptoms, which factors into the scoring severity index. It also gives an idea of the degree to which depression problems have affected an individual's functional level [25].

Outcome measures

This study was primarily designed to elucidate the IPs' effect on various psychoactive measures in habitual caffeine consumers. In addition, the probability of a combined effect to reduce the caffeine crash was explored by the coadministration of E-AG-01 and caffeine.

The primary efficacy variable was mental alertness, which is defined as achieving and maintaining a state of high sensitivity to incoming stimuli [26]. The alerting network is theorized to be responsible for achieving and maintaining vigilance and alertness while performing a continuous task. The Java version of the ANT 1.3.0 [27, 28] was used for this purpose, as it provides a behavioral measure of efficiency of the different components of the attention network separately within a single task. The participant was seated in a silent and secluded room. All external distractions were avoided and the participant was asked to give complete attention to the task at hand. Mental alertness in ANT was calculated in terms of the difference score quantified in milliseconds and calculated by subtracting the average double-cue response times (RTs) from the no-cue RTs. Higher scores indicate more efficient functioning of the alerting system.

The secondary efficacy variables included sustained attention (assessed by Psychomotor Vigilance Task [PVT]) and sleep architecture (assessed by Karolinska Sleepiness Scale [KSS] score, sleep duration measurement, Groningen's Sleep Quality Scale [SQS], and sleep diary].

Sustained attention to the environment is impacted by multiple underlying brain processes and related psychological constructs, out of which the sleep–wake state is an important aspect, and is dependent on multiple brain stem–thalamocortical pathways [29]. The PVT (according to the criteria reported by Basner et al. [30]) objectively assesses sustained attention, which might be associated with fatigue-related changes in alertness due to sleep loss, extended wakefulness, and circadian misalignment. Our study assessed the effect of the IP on the mean response time using a 10-min computer-based PVT. The mean reaction time (MRT) is the time taken by a participant to press the response button as soon as each stimulus appeared. Shorter MRT thus indicates more efficient and sustained attention.

The analysis of sleep architecture in this study comprised evaluation of effects on mental fatigue, wakefulness, and sleep patterns. Mental fatigue is a physiological state of reduced mental or physical performance resulting from sleep disturbances such as inadequate sleep, unwanted wakefulness, circadian disruption, or workload (mental and/or physical activity) that can impair an individual's alertness and ability to perform in a continuous task. It was assessed by the 10-point subjective KSS in terms of subjective sleepiness [31]. Lower scores on the scale imply low subjective sleepiness and hence less mental fatigue.

Participants were asked to complete the KSS on arrival at the site (baseline) and then prior to all sessions of ANT and PVT on all four visits.

Wakefulness is an important brain function, as its intensity might affect cognitive functions such as attention, memory, and decision making and for its assessment, participants were provided with a stopwatch to record duration of sleep between the two assessment sessions. Participants were asked to start the stopwatch before taking a nap and to stop it once they were awake or when they were woken up by the coordinator for an assessment. The stopwatch was lapped and restarted if the participant wanted to nap again.

Sleep patterns were assessed by Groningen's SQS questionnaire and a sleep diary [32]. In the SQS, sleep quality was graded in the form of a score on a scale of 0 to 14, and lower scores indicate higher subjective quality of sleep and vice versa. Participants were asked to complete the SQS questionnaire at all study visits prior to IP administration. In addition, the participants noted their time of going to bed and rising from the bed in the sleep diary.

Measurement of vital parameters and incidence of adverse events were recorded to ensure the safety of the IPs.

Power calculation

The trial was designed to demonstrate the efficacy of E-AG-01 over placebo to improve mental alertness. Based on the preliminary data, the sample size was calculated to detect the difference between E-AG-01 and placebo for the change in alertness score from baseline to 1, 3, or 5 hours after administration of the intervention. Based on the assumption of an intergroup score difference of 6 ± 1.5 ms (12% ± 3%), a sample size of 30 completed participants per group was calculated to maintain the study power at 90% with a type I error of 0.05. Further, accounting for dropouts and withdrawals (∼ 20%) as well as the nonevaluable participants for the primary efficacy outcome (∼25%), the study targeted to enroll 60 participants who were planned to cross over to each arm.

Statistical analysis

Statistical analysis was carried out using SPSS 15.0 for Windows (Chicago, IL, USA). Variables were tested for normality using the Shapiro-Wilk test. Chi-square test and student's unpaired *t*-test were applied while comparing different groups of responders for different independent variables. Student's paired *t*-test was used for intergroup comparisons at different time points. Multivariate analysis of variance was used to evaluate statistical significance among groups. Further, a repeated two-way analysis of variance (ANOVA) was performed to investigate the difference in alertness score due to time X treatment interaction. A p value <0.05 was considered statistically significant at the 95% confidence level. Participants who met all inclusion criteria and received at least one dose of each IP were considered the intent-to-treat population. However, because neurobehavioral functioning is heavily masked by the homeostatic drive for sleep and metabolism [33], the alertness score is expected to have interpersonal variability. Hence, final analysis was conducted on the per protocol population, wherein included participants had baseline alertness scores of 50 ± 20 ms at each individual study visit and completed the study visits successfully. The data were segregated based on the four interventional groups before subjecting them to statistical analyses and are represented in a similar manner in the results section.

Continuous variables (age, height, weight, and BMI) were summarized by treatment group using summary statistics (number of observations, mean, and standard deviation). ANOVA was applied to prove insignificance in demographic characteristics across the four groups. Data pertaining to PHQ-9 and GAD-7 parameters were also evaluated statistically by ANOVA for assessment of within-group significance level.

The effect size was calculated by Cohen's d method to determine clinical relevance of the observed effects in case of statistically significant outcomes [35].

Results

Study population

A total of 124 participants were screened for this study. Out of those, 59 met the protocol-defined inclusion criteria and were enrolled in the study. The participants enrolled in the study were predominantly right-handed [36], with a history of moderate caffeine consumption (2–4 cups of caffeinated beverages per day). Six of fifty-nine participants dropped out during the study, primarily due to withdrawal (4 of 6) or loss to follow-up (2 of 6). Two of the withdrawals were attributed to safety concerns. The study flowchart is depicted in Figure 1.

The varying number of participants in each group is a result of discarded data for those whose baseline parameters did not match the protocol-specified inclusion/exclusion criteria, and the excluded data did not affect the minimum study power of 90%.

Analysis of demographic characteristics of all participants confirmed the insignificance of the differences in demographics across the four groups. PHQ-9 and GAD-7 scores indicated the absence of a mental health condition such as anxiety or depression in the study population. Data pertaining to these measures are presented in Table 2.

Effect on primary outcome measures

Mental alertness was considered the primary outcome based on its importance in the assessment of cognitive performance. Data obtained from the ANT were expressed as the alertness score in milliseconds (Table 3 and Figure 2A). The baseline alertness scores were similar in all treatment groups.

In the placebo group, we observed a statistically insignificant increase in alertness score of 5.05 ± 19.71, 2.61 ± 20.66, and 9.79 ± 20.07 ms from baseline at 1, 3, and 5 hours, respectively. In the E-AG-01 group, we observed a significant increase in alertness score of 11.65 ± 23.94 (95% confidence interval [CI], −3.67–16.86, $p = 0.008$), 12.50 ± 19.73 (95% CI, 0.37–19.42, $p = 0.001$), and 12.62 ± 24.71 (95% CI, −7.69–13.35, $p = 0.005$) ms from baseline at 1, 3, and 5 hours, respectively. In the caffeine group, alertness scores compared to baseline increased significantly by 8.97 ± 18.20 ms (95% CI, −4.96–12.80, $p = 0.006$) at 1 hour; however, the score

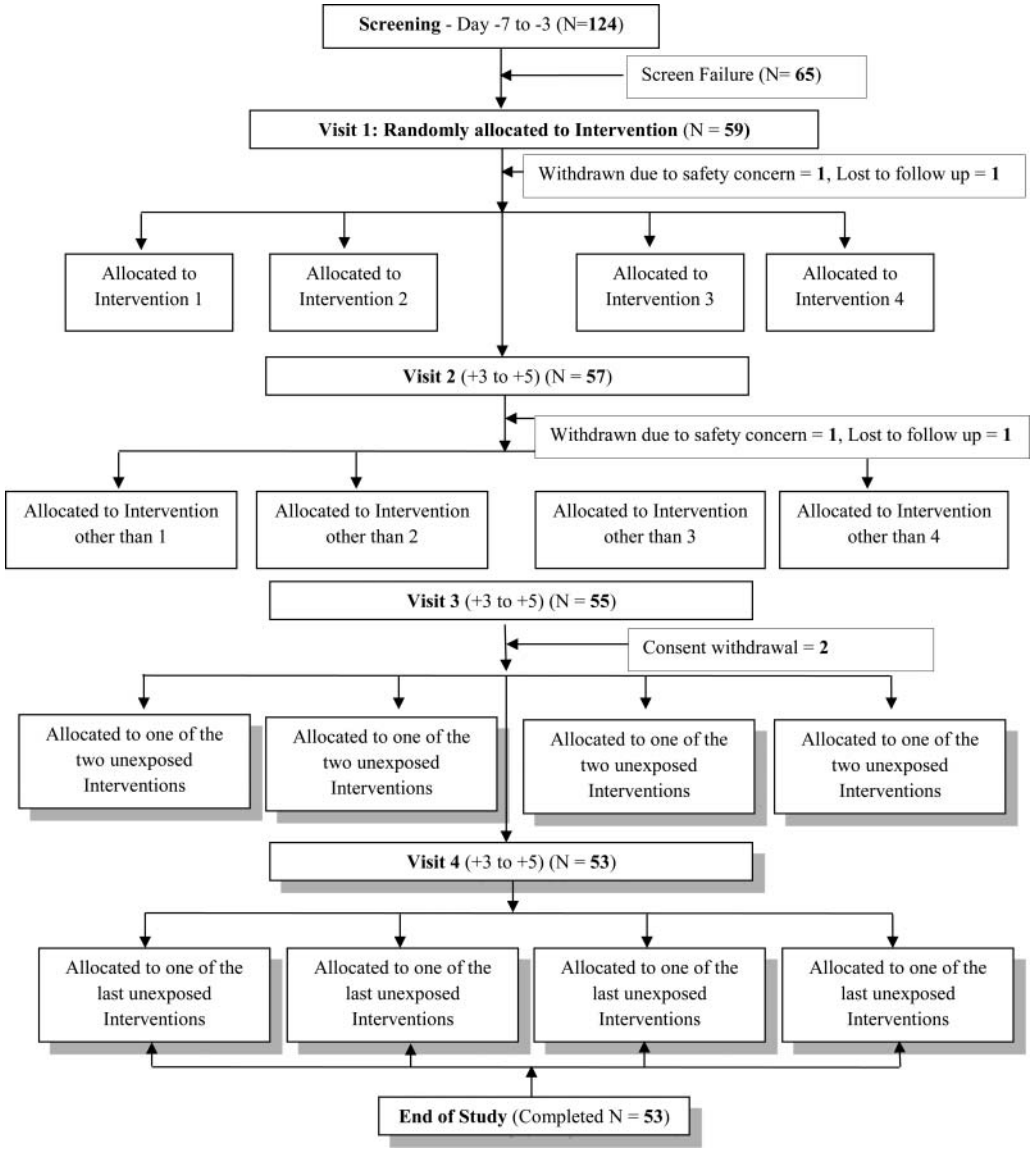

Figure 1. Study flowchart.

decreased by 1.23 ± 18.60 ms (95% CI, −13.04–5.37, $p = 0.698$) from baseline at 3 hours, indicating a caffeine crash. Also, in the composite group, scores significantly increased by 10.27 ± 20.34 ms (95% CI, −4.96–12.80, $p = 0.004$) from baseline at 1 hour, followed by a return approximately to the baseline score with a decrease of 0.68 ± 21.87 ms (95% CI, −13.01–6.43, $p = 0.074$) at 3 hours.

As the changes across the groups were significant at 3 hours ($p = 0.03$), all the groups were individually compared to the placebo group using student's paired *t*-test, wherein the

Table 2. Demographics and baseline characteristics.

Variables	Placebo (n = 38) Mean ± SD	E-AG-01 (n = 34) Mean ± SD	Caffeine (n = 35) Mean ± SD	Composite (n = 38) Mean ± SD	*p* Value
Male	19	17	16	19	—
Female	19	17	19	19	—
Age (years)	22 ± 0.5	22 ± 0.4	22 ± 0.4	22 ± 0.5	0.98
Height (m)	1.7 ± 0.1	1.7 ± 0.1	1.64 ± 0.1	1.7 ± 0.1	0.92
Weight (kg)	58.9 ± 9.2	59.7 ± 8.1	57.7 ± 8.2	58.5 ± 7.9	0.81
BMI (kg/m^2)	21.5 ± 2.0	21.6 ± 1.9	21.3 ± 1.9	21.3 ± 2.0	0.86
PHQ-9	1.5 ± 1.6	1.8 ± 1.6	1.5 ± 1.6	1.6 ± 1.6	0.92
GAD-7	1.9 ± 2.3	2.1 ± 2.1	1.5 ± 1.6	1.9 ± 2.3	0.66

E-AG-01 = *Alpinia galanga* proprietary extract; BMI = body mass index; PHQ-9 = Patient Health Questionnaire-9; GAD-7 = Generalized Anxiety Disorder-7.

Table 3. Effect of interventions on mental alertness with intergroup analysis.

ANT-Alertness Score in ms					
Variable	Placebo (n = 38)	E-AG-01 (n = 34)	Caffeine (n = 35)	Composite (n = 38)	*p* Value*
BL	49.6 ± 11.0	45.5 ± 11.2	48.0 ± 11.1	46.8 ± 12.0	0.45
1H	54.7 ± 17.0#	57.1 ± 23.8#	57.0 ± 18.1#	57.0 ± 21.7	0.94
3H	52.2 ± 19.5#	58.0 ± 21.2	46.8 ± 16.8	46.1 ± 19.1	0.03##
5H	59.4 ± 17.7#	58.1 ± 27.6	50.8 ± 19.0	54.2 ± 20.3#	0.31
Intergroup Analysis by Student's Paired *t*-test					
Variable		E-AG-01 vs Placebo	Caffeine vs Placebo	Composite vs Placebo	
p (1H)		0.20	0.38	0.26	
p (3H)		0.04###	0.40	0.50	
p (5H)		0.59	0.11	0.64	
Repeated Two-Way ANOVA for Time X Treatment Interaction					
Variable		E-AG-01 vs Placebo	Caffeine vs Placebo	Composite vs Placebo	
p (F)		0.26 (1.42)	0.73 (0.42)	0.63 (0.58)	

Note.
*Multivariate analysis of variance test applied across four groups to get *p* values.
**Paired *t*-test applied between two groups.
#Decreased significantly as compared to baseline.
##Statistically significant difference in score across the groups.
###Significant change in score as compared to placebo.
ANT = Attention Network Test; E-AG-01 = *Alpinia galanga* proprietary extract; BL = baseline value; 1H = value at 1 hour; 3H = value at 3 hours; 5H = value at 5 hours.

E-AG-01 group demonstrated a statistically significant improvement in alertness score ($p = 0.04$).

At 5 hours, all groups demonstrated an increase in alertness score, owing to the logistic factors of the study and asymptomatic performance improvement due to the diurnal pattern of alertness [37]; however, the increase in alertness score was greatest in the E-AG-01 group (12.62 ± 0.68 ms from baseline).

As the result was statistically significant for E-AG-01, we calculated the effect size in terms of Cohen's *d* value in comparison with placebo. A value between 0.20 and 0.49 was considered a small effect, a value between 0.5 and 0.79 was considered a medium effect, and ≥0.8 indicated a large effect. The derived value of $d = 0.59$ for the E-AG-01 group against $d = 0.08$ for the caffeine group confirmed a significant medium effect in alertness score in the E-AG-01 group compared to a remarkably small effect in caffeine group.

A time X treatment interaction effect was analyzed using a repeated two-way ANOVA, and the results indicated that there was no significant effect of this particular interaction on the effect of IP in any of the three groups as compared to placebo (Table 3). These results also confirmed the absence of an influence of any carryover effect in this clinical study of crossover design. Hence, the data from all treatment groups in the different time periods were analyzed in a usual manner.

Effect on secondary outcome measures

This study assessed the effect of IP on sustained attention by using the PVT, and results were expressed as MRT in milliseconds in Table 4 and Figure 2B.

As evident from the results, the E-AG-01 group did not demonstrate any significant improvement in MRT, whereas the results in the caffeine group suggested a declining trend in MRT until 3 hours, after which it showed an increase, which may be attributed to the "crash" effect. The composite group exhibited a trend similar to the caffeine group but achieved a within-group statistical significance at 5 hours interval as compared to baseline ($p = 0.02$). In addition, intergroup analysis for the composite group revealed a significant decrease in MRT

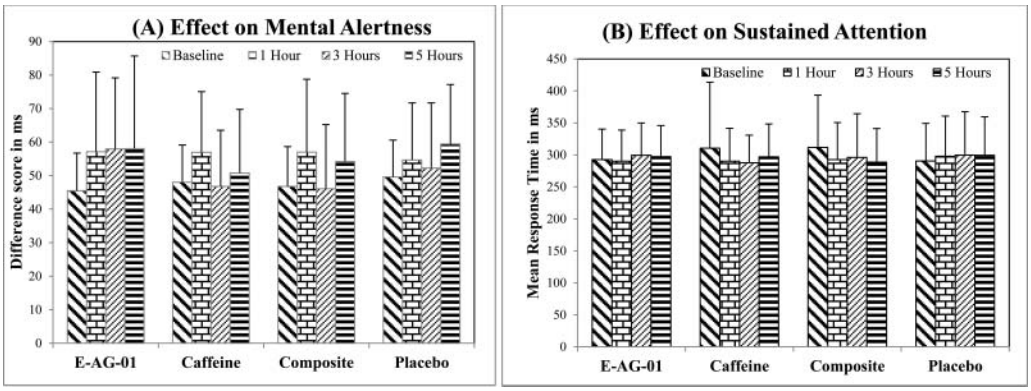

Figure 2. Effect of interventions on (A) mental alertness and (B) sustained attention.

Table 4. Effect of interventions on sustained attention with intergroup analysis.

PVT–Mean Response Time in ms					
Variable	Placebo(n = 38)	E-AG-01 (n = 34)	Caffeine(n = 35)	Composite(n = 38)	*p* Value*
BL	290.4 ± 59.0	292.7 ± 47.7	310.4 ± 103.3	311.6 ± 81.9	0.50
1H	297.9 ± 62.6	290.2 ± 48.5	290.1 ± 51.6	292.7 ± 58.0	0.92
3H	299.7 ± 67.8	299.5 ± 50.2	287.6 ± 43.1	296.0 ± 68.3	0.82
5H	299.3 ± 60.3	297.1 ± 48.8	296.9 ± 51.5	288.7 ± 52.6#	0.84
Intergroup Analysis by Student's Paired *t*-test**					
Variable		E-AG-01 vs. Placebo	Caffeine vs. Placebo	Composite vs. Placebo	
p (1H)		0.13	0.09	0.01###	
p (3H)		0.73	0.06	0.04###	
p (5H)		0.60	0.17	0.01###	

Note:
*Multivariate analysis of variance test applied across four groups to get *p* values.
**Student's paired *t*-test applied between two groups.
#Decreased significantly as compared to baseline.
###Significant change in score as compared to placebo.
PVT = Psychomotor Vigilance Task; E-AG-01 = *Alpinia galanga* proprietary extract; BL = baseline value; 1H = value at 1 hour; 3H = value at 3 hours; 5H = value at 5 hours.

compared to the placebo group at 1 hour ($p = 0.01$), 3 hours ($p = 0.04$), and 5 hours ($p = 0.01$).

The data on various parameters of sleep architecture revealed the statistically insignificant influence of IP on mental fatigue, wakefulness, and sleep pattern.

Effect on safety measures

The safety of the IPs was determined by analyzing the vital parameters (heart rate [HR] and pulse rate [PR]) and occurrence of the adverse events. This study demonstrated a very good safety profile with clinically insignificant safety concerns. One of the fifty-nine enrolled participants was withdrawn due to persistent hypertension (systolic blood pressure >140 mm Hg for 3 consecutive days) and another participant was withdrawn due to reported giddiness at the end of the clinic stay. No unblinding was done at that time, but decoding of the arms at the statistical analysis stage revealed the product dispensed to be *A galanga* in the former case and caffeine in the latter. The occurrence of these adverse events may be remotely attributed to confounding factors of the study such as a specific hypersensitivity to the allocated interventions. No other case of hypertension or tachycardia was reported in any of the groups.

Discussion

The present study examined the effect of *A galanga* (E-AG-01) in comparison with caffeine (a comparator) and a placebo (a control) on attention network by ANT. Consistent with caffeine's well-known effect on the alerting network, alertness scores increased until 1 hour, followed by a reduction—probably due to a caffeine crash—at 3 hours. At the same time, E-AG-01 showed an improvement in alertness scores until 5 hours. In the composite group, alertness scores increased significantly at 1 hour followed by a reduction, indicating a caffeine crash, which was less than that observed in the caffeine group. Hence, it can be hypothesized that the E-AG-01 is able to impede the caffeine crash, as evident from 3-hour and 5-hour alertness scores.

The alerting network recruits a distributed network of brain regions, primarily the thalamus and bilateral frontal and parietal brain regions [38]. Given the dense dopaminergic innervation of the human thalamus and prefrontal cortex [39] and that caffeine is generally thought to up-regulate dopaminergic availability [40], the present results are consistent with the theorized effects of caffeine on CNS function. Based on these facts, it can be postulated that E-AG-01 also improves alertness in a similar way as caffeine, by enhancing dopaminergic activity.

The effect size for this efficacy parameter was calculated in terms of standardized mean effect (denoted as Cohen's *d*), which expresses the mean difference between two groups in standard deviation units. The results suggest that in terms of probability of superiority of treatments, there is a 66% greater chance that a randomly selected participant from the E-AG-01 group will exhibit a definite improvement in mental alertness than a randomly selected participant from the placebo group.

Alternatively, it can be stated that the effect size represented as Cohen's *d* value indicates that 73% of participants from the E-AG-01 group ($d = 0.599$) would exhibit greater alertness than the participants in the placebo group as compared to the caffeine group, wherein only 47% of participants would have greater alertness.

Sustained attention was assessed by the PVT, which generally reflects the arousal and attention state of an individual. Caffeine appears to exhibit dose-dependent performance improvement in a variety of basic psychomotor tasks as a direct result of altered CNS activity and is well reported by a number of studies [41–42]. Some studies also suggest that extended vigilance is generally improved following caffeine consumption at a dose of ~400 mg [43] and performance diminishes with very high dose of caffeine (e.g., 600 mg) [44]. In agreement with these reported findings, in this study neither caffeine at 200 mg nor E-AG-01 at 300 mg independently resulted in significant reductions in MRT. However, the combination of these ingredients at the same dose was found to be effective in improving sustained attention, as indicated by statistically significant data obtained in the composite group. It has been reported that the relationship between sustained attention and task performance

follows an inverted U-curve, i.e., poor performance can occur due to both under- and over-arousal. [45] This can be one of the reasons for a widespread range of observations in MRT, leading to greater standard deviations and insignificant *p* values. However, derived *p* values showed a positive trend in the reduction of MRT in the caffeine and composite groups. Hence, an attempt was made to analyze the treatment groups individually in comparison with placebo by student's *t*-test, which showed that the improvement in sustained attention was statistically significant in comparison with placebo, implying the superiority of the composite intervention in enhancing sustained attention and arousal state. Thus, we can hypothesize that coadministration of E-AG-01 with caffeine modulates neural activity in the cerebral regions related to sustained attention.

We attempted to analyze the effect of E-AG-01 with and without caffeine on sleep architecture. The KSS is well correlated with subjective sleepiness, with a very strong linear relation and extremely small standard errors when analyzed by ANOVA [46], thus imparting considerable reliability and concurrent validity to the tool. Effect of caffeine on KSS has been demonstrated in a clinical trial [47]. We did not encounter any significant reduction in mental fatigue in any of the experimental groups.

The derived data for wakefulness as measured by midday nap duration could not provide any significant information about the effect of the IPs on wakefulness in comparison with placebo. Sleep architecture as studied by Groningen's SQS, and sleep diaries could not dispense any distinguishable findings to substantiate the higher efficacy of E-AG-01 or caffeine or composite intervention over placebo. This finding can be correlated with the fact that the study population included the participants habituated to caffeine intake, due to which no remarkable alterations in sleep patterns were observed across the study groups. Hence, we concluded that the IP did not demonstrate any significant effect on sleep architecture, and we propose that more sensitive and tangible methods such as polysomnography may be useful in prospective studies to have an adequate qualitative and quantitative assessment of the sleep structure.

Clinical safety of these IPs was assessed by the measurement of vital parameters and recording adverse events during the study period. The data revealed no major safety concerns related to intake of *A galanga*.

Future recommendations

Effects of *A galanga* on sleep architecture can be further evaluated by high-sensitivity tools such as polysomnography to affirm its use in the normal population requiring adequate alertness to perform in day-to-day life. Although this research on the benefits of E-AG-01 is promising for mental alertness, ingredients belonging to the category of psychostimulants are prone to exhibit habituation. Hence, the addictive potential of *A galanga* needs to be analyzed. In addition, analogous to caffeine, the empirical data suggest that these types of ingredients have an interactive effect on cognitive performance. This suggests an objective for a future study to explore the long-term effect of *A galanga* consumption in domains related to cognitive processes such as working memory [48].

Conclusions

This is the first study in humans to demonstrate the psychostimulant effect of *A galanga* in comparison with caffeine and placebo on some of the important aspects of attention. The statistically significant results serve as constructive evidence for the beneficial effect of E-AG-01 on enhancement of mental alertness and sustained attention. Thus, this study was an effort to confirm the acute psychostimulant benefits of *A galanga*, rendering it a novel energy ingredient for day-to-day use. In summary, these findings support *A galanga*'s potential to create a significant impact on real-life work performance with an absolute requisite of mental alertness for populations such as drivers, video-gamers, college students, and athletes, who always strive to perform better.

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Author contributions

The study was conceptualized and designed by Dr Shalini Srivastava in coordination with Dr Mark S. Mennemeier. The manuscript was prepared and refined through collective efforts of all the listed authors. Dr Shalini Srivastava agrees to be accountable for all aspects of the work to ensure that the questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Dr Mark S. Mennemeier has been extensively involved in revising the manuscript critically for important intellectual content. Dr Surekha Pimple was substantially involved in the manuscript preparation. All authors read and approved the final manuscript for publication.

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