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Current Research in Complementary & Alternative Medicine

Bloomer RJ, et al. Curr Res Complement Altern Med. CRCAM-134.

Research Article

DOI:10.29011/ 2577-2201 /100034

Effects of a Single Dose of Teacrine®, Caffeine, or their Combination on Subjective Feelings, As Well as Heart Rate and Blood Pressure in Adults

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Citation: Bloomer RJ, Butawan M, Stockton ML, Smith NJG (2018) Effects of a Single Dose of Teacrine®, Caffeine, or their Combination on Subjective Feelings, As Well as Heart Rate and Blood Pressure in Adults. Curr Res Complement Altern Med. CRCAM-134. DOI:10.29011/ 2577-2201 /100034

Received Date: 07 December, 2018; **Accepted Date:** 14 December, 2018; **Published Date:** 21 December, 2018

Abstract

Background: Theacrine is structurally similar to caffeine, and reported to improve mood and cognition in human subjects. The purpose of the present study was to determine the impact of theacrine on subjective mood, cognitive performance, Heart Rate (HR) and Blood Pressure (BP).

Methods: 24 men (aged: 24.3±6.1) and 26 women (aged: 23.4±3.5) ingested a placebo, theacrine (Teacrine®, Compound Solutions, Inc.) at 25mg, at 125mg, caffeine at 150mg, or theacrine at 125mg + caffeine at 150mg on five separate occasions, separated by approximately one week. HR and BP were measured before ingestion and at 30 minutes, 1, 2, 3, 4, and 5 hours post ingestion. Subjects rated their subjective feelings at the above times, and performed the Trail Making Test (TMT) of cognitive performance at baseline and at hours 2 and 4 post ingestion.

Results: Treatment with theacrine had little impact on HR and BP, with only marginal increases noted (~3 bpm; ~3mm Hg). No condition effects were noted for the TMT ($p>0.05$), with similar scores noted for all conditions. Effects were noted for subjective feelings such as attentive, focus, energy, and motivation to exercise; with the caffeine and theacrine+caffeine generally noted to have the most favorable scores, with theacrine at 25mg and placebo generally noted to have the least favorable scores.

Conclusion: These findings indicate that theacrine, when used alone at 125mg or in combination with caffeine, does not result in any significant increase in HR or BP but may improve measures of subjective feelings in healthy men and women.

Keywords: Blood Pressure; Caffeine; Mood; Theacrine; Trail Making

Introduction

The use of dietary supplements to increase energy and focus is commonplace, as evidenced by the widespread sale and consumption of energy drinks and energy shots, as well as capsule-based dietary supplements. Recently, a novel plant-based ingredient has made its way into the dietary supplement market: a purine alkaloid found primarily in the leaves of the Camellia Kucha plant known as theacrine [1].

Theacrine (1,3,7,9-tetramethyluric acid) is structurally like caffeine and has been reported to have antioxidant [2], anti-inflammatory/analgesic [3], anti-depressive [4], locomotor [5], and sedative/hypnotic properties [6]. Collectively, this agent may have wide-ranging effects in human subjects and has been evaluated in three published studies to date. The initial two-part pilot study involving human subjects administered theacrine at 200 or 400 mg in 9 subjects over a 7-day period. Researchers noted moderate to large effect sizes for energy, fatigue, concentration, anxiety, motivation to exercise, and libido with the 200-mg dose [7]. The same study included data for a group of 15 subjects who

were acutely dosed 200 mg of theacrine where they reported a significant increase in energy, a reduction in fatigue, and a trend (p=0.07) towards improved concentration (based on a subjective questionnaire) compared to a placebo group. In a more recent study involving human subjects, 60 young and healthy men and women received either a placebo or theacrine at a dosage of 200 or 300 mg per day for a period of eight weeks to determine the safety profile of this agent in human subjects. No changes of statistical significance were noted for any safety measure [8].

We recently studied the combination of caffeine (150mg) and theacrine (100mg) in 20 healthy men and women [9]. Before, and for up to 4 hours following, ingestion of the assigned condition, subjects completed a subjective assessment of energy and mood, as well as tests of cognitive performance (trail making test, digit symbol substitution test), and reaction time. Heart rate and blood pressure were measured. No significant effects were noted for cognitive performance or reaction time, despite a trend for improvement in selected variables with treatment. Values for attentive, alert, focused, and energetic were higher for theacrine/caffeine condition as compared to placebo and caffeine alone, while values for lethargic and groggy were lower for the theacrine/caffeine condition. Heart rate and blood pressure were largely unaffected by treatment (e.g., heart rate was 3-5bpm lower in the hours following treatment; blood pressure was 1-2mmHg higher in the hours following treatment) and subjects did not report any adverse outcomes related to ingestion of the theacrine/caffeine. That said, because both theacrine and caffeine are viewed as stimulants, it is possible that blood pressure and heart rate may be elevated in selected individuals following intake, while such individuals may also experience symptoms such as shakiness and irritability. Further work is needed to confirm the safety profile of theacrine when used alone and in conjunction with caffeine. Moreover, additional data are needed to support the preliminary findings of improved subjective mood with theacrine use. The present study was designed to determine the impact of theacrine, alone and in combination with caffeine, on heart rate, blood pressure, subjective feelings, and cognitive performance in a sample of 50 healthy men and women.

Materials and Methods

Subjects

A total of 24 men and 26 women participated in this study. Subjects were recruited via word of mouth conversations, formal presentations discussing study participation, online postings, and recruitment flyers posted on and off campus. Health history, medication and dietary supplement usage, and physical activity questionnaires were completed by all subjects and reviewed in detail by an investigator to determine eligibility. Subjects were not self-reported smokers and were in good overall health based on a review of their self-reported health history. They did not have a history of cardiovascular, neurological, or metabolic disorders (e.g., hypertension, seizures, and diabetes). Subjects were regular consumers of stimulants (e.g., caffeine) within beverages or nutritional supplements, who did not report a history of adverse reactions to caffeine or other stimulants.

Prior to participation, each subject was informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form. The study procedures were approved by the University Institutional Review Board for Human Subjects Research (approval document number 4021) and subjects provided written informed consent prior to participating. Subjects who completed the study protocol were compensated $250 for their time and effort.

Initial Laboratory Visit: screening visit

During the initial visit to the laboratory, subjects completed the informed consent form, health history, and physical activity questionnaires. Following a 10-minute rest period, subjects' heart rate and blood pressure, height, weight, waist and hip circumference were measured. The above data were used for descriptive purposes and subject characteristics are presented in (Table 1). Female subjects were required to take a urine pregnancy test. Upon completion of the screening and verification that subjects met all inclusion criteria, subjects were scheduled for their initial testing visit.

Variable	Men (n=24)	Women (n=26)	P value
Age (years)	24.3±6.1	23.4±3.5	0.50
Height (cm)	180.1±6.6	165.9±5.4	0.00
Body Weight (kg)	86.0±13.2	66.5±13.8	0.00
Body Mass Index ($kg \cdot m^{-2}$)	26.4±3.3	24.2±5.1	0.07
Waist Circumference (cm)	87.8±8.3	79.1±11.0	0.00
Hip Circumference (cm)	104.9±7.2	101.7±11.4	0.25
Waist: Hip	0.84±0.04	0.78±0.06	0.00
Heart Rate (bpm)	63.8±8.6	73.1±11.0	0.00
Systolic Blood Pressure (mmHg)	117.8±4.7	113.6±8.0	0.03

Diastolic Blood Pressure (mmHg)	67.2±7.0	70.6±8.4	0.12
Weekly Aerobic Training (hrs)	2.1±1.6	2.1±1.8	0.92
Aerobic Training History (yrs)	6.7±6.7	5.2±5.5	0.38
Weekly Anaerobic Training (hrs)	2.8±2.1	1.0±1.2	0.00
Anaerobic Training History (yrs)	6.1±7.6	3.3±4.7	0.12
Daily Caffeine Intake (mg)	108.6±85.1	96.9±75.8	0.61
Values are mean±SD			

Table 1: Characteristics of men and women.

Independent Variable

The dietary ingredient known as theacrine was evaluated, alone and in conjunction with caffeine, in addition to a placebo condition (a total of five condition combinations). Since both theacrine and caffeine are used in combination within dietary supplements, it was of interest to know whether the combination of these ingredients had a differing impact on the outcome measures as compared to each ingredient in isolation. All capsules were produced in accordance with Good Manufacturing Practices and were of similar appearance. Subjects ingested the assigned condition on each of the five test days in the presence of an investigator. Only one capsule was ingested each test day.

The following conditions were included and assigned in a double-blind manner: placebo (microcrystalline cellulose), 25 mg theacrine; 125 mg theacrine; 150 mg caffeine; 125 theacrine + 150 mg caffeine. The theacrine used in this study was provided by Compound Solutions, Inc. (Teacrine®). Caffeine was delivered as caffeine anhydrous at 150 mg.

Test Visit Procedures

Subjects reported to the lab a total of five times during the morning hours. Testing began between approximately 8:00am and 9:00am. Subjects reported in a 10-hour fasted state, without having consumed alcohol or caffeine within the past 48 hours. Subjects were instructed to obtain at least 7 hours of sleep during the night prior to testing. Subjects should not have performed strenuous physical exercise within the past 24 hours. The time of day was matched for each lab visit and approximately 7 days separated each visit.

Upon arrival, subjects received a standardized meal (250 calories), consisting of a meal replacement shake. Testing began 30 minutes later. Resting heart rate and blood pressure were obtained pre-ingestion of the conditions and at 30 minutes, 1, 2, 3, 4, and 5 hours post ingestion using an automated unit (Omron HEM 907XL) with duplicate measures taken at each time. Rate pressure product was calculated (heart rate x systolic blood pressure).

Cognitive performance (using the Trail Making Test (TMT); versions A and B) was assessed pre-ingestion and at hours 2 and 4 post ingestion. The TMT was used as an assessment of cognitive performance, focus, and attentiveness [10]. This test provides a numeric score (time taken to complete the task in seconds) which can then be used as an objective indicator of condition effectiveness. Practice trials were administered to subjects during the screening visit, as some learning effects are expected [11]. Different versions of the test were provided to subjects at all assessment times and attempts were made to provide equal difficulty for all tests by using similar spacing and locations between consecutive numbers and letters Subjects completed parts A and B of the test. This included connecting numbers only (part A) or numbers and letters (part B) from beginning to end (from low to high; 1-13 and A-L), as quickly as possible. Subjects were provided with detailed instructions to alternate their tracing pattern between a number and letter in the proper order. If a mistake was made, investigators stopped the participant, instructed them to go back to the circle where the mistake was made, and continue the test. Time to completion represents the total time (in seconds) for each testing trial. This test was used in our prior study investigating theacrine and caffeine use [9].

Due to the potential influence of herbal supplements on subjective feelings, subjects completed a questionnaire pertaining to mood, energy, and related variables using a 10cm Likert scale always, with 10 representing the highest rating. We have used a near identical form in a recent study of theacrine and caffeine [9]. These data were helpful to understand how participants felt at selected times during the study period. A standardized meal (meal replacement shake) was provided to subjects following all data collection at hour 5.

Dietary Intake and Physical Activity

All subjects were instructed to consume their usual diet throughout the study period. During the day prior to each test day, subjects recorded all food and drink consumed and attempted to mimic this intake for the day prior to subsequent visits. Diet records were analyzed using nutrient analysis software (Food Processor SQL, version 9.9; ESHA Research, Salem, OR). For the actual test days, ready-to-drink shakes [Orgain Organic Nutrition™]) were provided to subjects at the times indicated above. Each shake contained 250 calories, 7 grams of fat, 32 grams of carbohydrate, and 16 grams of protein. No food other than what was provided to subjects was allowed during each study period. The only beverage that subjects could consume during the study period was water and the volume consumed while in the lab was matched for each test

day (men: 41±4 ounces; women: 32±2 ounces). Physical activity was to remain similar for subjects throughout the study period, except for refraining from strenuous physical activity during the 24 hours prior to each test day.

Data Analysis

The data are presented as mean ± SD (descriptive characteristics) or mean ± SEM. Data were initially analyzed using a 5 (condition) x 2 (gender) x 7 (time) Analysis of Variance (ANOVA), with Tukey post hoc testing as appropriate. Note: only three times were used in the analysis of TMT data. Statistical significance was set at $p \leq 0.05$. IBM-SPSS Statistics 24.0 was used for the following analyses. One-way repeated measures ANOVAs were conducted in an a priori manner to compare the effect of each condition on cognitive performance, attention, focus, energy, and motivation to exercise across the respective times. A Greenhouse-Geiser correction was applied to the degrees of freedom when reporting the ANOVA results because the assumption of sphericity was not met for the following variables: attention, focus, energy, and motivation to exercise. A Bonferroni adjustment was used with all pairwise comparisons to keep the Type I error at 5% overall.

Results

Overview

All 50 subjects successfully completed the study. No subject reported a significant adverse event, or any other problems associated with the ingestion of the conditions. Subject unsolicited comments indicated that the treatments were well-tolerated. No concerns were raised that required adverse event reporting to the IRB. As expected, many differences existed between men and women for descriptive variables. Descriptive data are reported in (Table 1), with p values included for each variable.

Dietary Data

Pertaining to dietary intake, differences existed for men and women with regards to the intake of kilocalories ($p<0.0001$), protein ($p<0.0001$), carbohydrate ($p=0.0001$), fiber ($p=0.007$), sugar ($p=0.0003$), fat ($p<0.0001$), vitamin C ($p=0.05$), and vitamin E ($p<0.0001$). Aside from gender differences in dietary intake, dietary data were not different during the days prior to each test visit ($p>0.05$). Data are provided in (Table 2).

Variable	Theacrine 25mg	Theacrine 125mg	Caffeine 150mg	Theacrine+Caffeine 125mg+150mg	Placebo
MEN					
Kilocalories	2470.6±212.7	2476.3±178.5	2463.2±193.3	2270.9±164.1	2633.5±213.6
Protein (g)	116.9±15.2	114.7±13.2	122.0±16.9	111.2±15.2	122.1±15.2
Carbohydrate (g)	285.8±29.1	294.4±27.5	281.4±29.7	260.9±23.3	303.3±29.1
Fiber (g)	21.8±3.1	25.1±3.2	22.9±2.8	20.6±2.5	25.0±3.1
Sugar (g)	115.9±14.1	104.8±14.0	101.2±13.9	85.5±9.4	98.2±14.1
Fat (g)	98.7±10.1	96.6±9.9	98.0±8.8	90.4±7.6	105.8±10.1
Vitamin C (mg)	115.3±38.4	129.4±37.7	122.6±41.0	92.9±18.4	106.9±38.4
Vitamin E (mg)	9.7±3.3	10.7±3.1	8.7±2.4	9.1±2.1	10.8±3.3
Vitamin A (RE)	5442.0±1475.5	5821.5±1670.2	6064.7±1691.3	9982.4±3725.7	5714.6±1721.1
WOMEN					
Kilocalories	1805.1±91.7	1729.9±101.3	1844.8±84.2	1856.9±108.1	1798.6±96.1
Protein (g)	77.8±6.3	68.0±5.1	77.9±6.0	77.7±9.5	71.2±5.3
Carbohydrate (g)	213.8±12.6	206.0±12.5	225.1±12.7	221.0±11.5	216.4±11.3
Fiber (g)	19.8±2.1	18.2±1.5	19.4±2.1	18.1±1.6	19.1±1.6
Sugar (g)	72.5±7.4	74.1±7.4	73.3±8.6	73.4±7.3	82.8±7.1
Fat (g)	74.2±5.6	73.9±6.9	77.5±5.6	75.5±6.8	76.1±6.4
Vitamin C (mg)	92.1±16.8	78.7±15.0	79.7±16.3	68.6±13.5	84.7±15.4

Vitamin E (mg)	4.2±1.0	4.9±1.0	7.8±1.1	4.8±0.9	4.8±1.1
Vitamin A (RE)	10193.3±1721.1	7526.9±1903.8	8224.7±2273.4	6076.5±1941.9	5708.0±1176.7
Values are mean ± SEM					

Table 2: Dietary data of men and women during the 24 hours prior to each test day.

Heart Rate and Blood Pressure

With regards to heart rate (HR, (Table 3)), systolic and diastolic blood pressure (SBP and DBP, (Tables 4,5), respectively), and rate pressure product (RPP, (Table 6)), the following was noted: First and as expected, gender differences existed for HR ($p<0.0001$), SBP ($p<0.0001$), DBP ($p<0.0001$), and RPP ($p<0.0001$).

- A condition effect was noted for HR, with theacrine 125mg higher than caffeine ($p<0.05$). A time effect was also noted for HR, with hours 3, 4, and 5 lower than pre, 0.5, and 1 hours ($p<0.05$). No other effects were noted for HR ($p>0.05$).
- A condition effect was noted for SBP, with caffeine and theacrine+caffeine higher than the other three conditions ($p<0.05$). A time effect was also noted for SBP, with all times post ingestion higher than pre-ingestion ($p<0.05$). No other effects were noted for SBP ($p>0.05$).
- A condition effect was noted for DBP, with caffeine and theacrine+caffeine higher than the other three conditions ($p<0.05$). A time effect was also noted for DBP, with all times post ingestion higher than pre-ingestion ($p<0.05$). No other effects were noted for DBP ($p>0.05$).
- A time effect was noted for RPP, with 0.5 hours higher than pre, 2, 3, 4, and 5 hours post ingestion ($p<0.05$). No other effects were noted for RPP ($p>0.05$).

	Theacrine 25mg	**Theacrine 125mg**	**Caffeine 150mg**	**Theacrine+Caffeine 125mg+150mg**	**Placebo**
MEN					
Pre	64.5±2.1	65.8±2.0	60.1±2.1	63.6±1.8	63.1±2.1
0.5hr	64.7±2.0	66.2±2.0	61.5±2.2	66.2±2.4	65.4±2.2
1hr	63.7±2.4	64.2±1.9	71.8±2.1	64.1±2.3	64.3±2.1
2hr	60.2±2.1	63.8±2.3	60.6±2.1	61.8±2.5	62.0±2.4
3hr	58.3±1.9	61.3±2.1	60.1±1.9	61.5±2.3	60.9±2.4
4hr	60.1±1.9	61.5±2.0	59.4±2.0	61.2±2.1	59.9±2.2
5hr	60.2±2.1	62.6±1.9	59.6±1.9	62.2±2.3	60.6±2.0
WOMEN					
Pre	73.2±2.1	73.8±2.0	72.4±2.4	71.2±2.1	73.9±1.9
0.5hr	75.3±2.2	76.7±1.8	74.3±1.9	74.0±2.0	75.1±1.6
1hr	73.6±2.2	73.8±2.4	72.6±2.0	72.1±2.0	74.4±1.8
2hr	71.5±2.1	71.6±2.3	68.7±2.2	70.6±1.7	71.4±2.0
3hr	70.5±2.3	69.0±2.1	67.2±2.1	69.0±1.9	70.0±1.9
4hr	72.2±2.8	69.7±1.8	68.5±1.9	66.8±1.7	70.8±2.0
5hr	70.3±2.2	70.0±2.0	68.1±1.9	68.9±2.0	71.3±1.7
Values are mean ± SEM					

Table 3: Heart rate (bpm) data of men and women ingesting theacrine and/or caffeine.

	Theacrine 25mg	Theacrine 125mg	Caffeine 150mg	Theacrine+Caffeine 125mg+150mg	Placebo
MEN					
Pre	117.8±1.7	116.8±1.5	118.7±0.9	118.1±1.3	117.0±1.2
0.5hr	119.0±1.5	118.1±1.7	124.8±1.3	124.8±1.5	118.9±1.5
1hr	117.8±2.4	119.0±1.7	126.2±1.7	125.3±1.8	120.0±1.3
2hr	119.3±2.1	118.0±1.5	126.0±1.0	125.5±2.0	118.6±1.2
3hr	117.8±1.9	119.2±1.9	122.3±1.5	122.3±1.7	120.3±1.6
4hr	117.9±1.9	119.8±1.5	123.5±1.2	120.7±1.7	120.1±1.8
5hr	119.9±2.1	120.6±1.3	122.5±1.5	122.5±1.6	119.0±1.4
WOMEN					
Pre	113.7±1.7	113.9±1.4	112.2±1.5	113.3±1.7	112.1±1.8
0.5hr	116.2±1.6	116.7±1.5	119.5±1.7	119.3±1.5	115.2±1.8
1hr	117.1±1.7	117.0±2.2	120.8±2.2	120.2±1.8	115.3±1.7
2hr	116.0±2.2	116.0±1.4	118.1±1.9	117.8±1.8	115.6±1.8
3hr	115.3±1.6	115.9±2.1	116.5±2.1	116.6±1.6	115.8±1.9
4hr	114.7±1.6	115.5±1.9	115.4±2.4	117.2±1.6	117.2±1.7
5hr	115.4±1.7	116.7±2.2	117.5±2.1	115.3±1.7	114.8±1.7
Values are mean ± SEM					

Table 4: Systolic blood pressure (mm Hg) data of men and women ingesting theacrine and/or caffeine.

	Theacrine 25mg	Theacrine 125mg	Caffeine 150mg	Theacrine + Caffeine 125mg+150mg	Placebo
MEN					
Pre	65.8±1.7	66.6±1.5	67.4±1.5	65.8±1.5	66.5±1.5
0.5hr	68.9±1.8	68.4±1.6	73.1±1.1	72.8±1.6	70.0±1.4

1hr	69.3±1.6	69.6±1.2	75.5±1.3	76.0±1.7	69.8±1.5
2hr	70.7±1.5	70.9±1.7	75.7±1.4	76.2±1.8	70.4±1.6
3hr	70.0±1.7	69.9±1.3	73.8±1.3	74.5±1.5	71.2±1.6
4hr	70.0±1.6	70.7±1.5	73.5±1.6	73.2±1.8	71.6±1.7
5hr	69.8±1.7	71.0±1.5	73.3±1.3	73.5±1.7	70.7±1.3
WOMEN					
Pre	67.4±1.4	69.5±1.8	68.9±1.4	69.5±1.8	68.8±1.5
0.5hr	72.1±1.4	71.7±1.8	76.2±1.3	75.8±1.9	71.2±1.5
1hr	72.2±1.3	73.1±1.8	76.3±2.0	77.4±2.0	71.4±1.7
2hr	73.8±2.0	74.8±1.6	73.8±1.8	77.8±1.7	72.5±1.6
3hr	71.7±1.6	74.8±1.8	75.3±1.9	75.5±1.8	70.5±1.7
4hr	72.0±1.6	71.8±1.6	74.8±1.9	75.8±1.6	71.9±1.4
5hr	72.7±1.8	73.0±1.8	73.8±2.2	73.6±1.7	70.6±1.5
Values are mean±SEM.					

Table 5: Diastolic blood pressure (mm Hg) data of men and women ingesting theacrine and/or caffeine.

	Theacrine 25mg	**Theacrine 125mg**	**Caffeine 150mg**	**Theacrine+Caffeine 125mg+150mg**	**Placebo**
MEN					
Pre	7637.1±303.9	7703.5±282.1	7127.8±254.5	7518.6±233.6	7398.3±265.3
0.5hr	7729.0±294.8	7848.2±315.3	7696.9±304.9	8268.8±330.5	7779.8±284.9
1hr	7524.9±322.3	7649.3±265.2	7787.7±277.8	8041.4±329.2	7723.5±284.7
2hr	7210.4±312.1	7535.5±296.4	7634.4±275.5	7765.4±336.7	7366.0±316.8
3hr	6883.6±275.9	7315.9±299.5	7349.3±233.1	7494.8±279.3	7327.5±308.3
4hr	7102.3±272.8	7383.9±284.7	7334.0±260.8	7350.6±235.8	7209.2±295.8
5hr	7229.4±280.4	7557.6±262.2	7300.5±248.0	7614.6±299.9	7224.6±258.8
WOMEN					
Pre	8356.2±303.0	8424.9±271.6	8147.0±311.9	8112.1±309.8	8320.7±291.2

0.5hr	8767.3±310.2	8954.5±255.6	8892.8±278.5	8817.9±261.0	8668.3±257.7
1hr	8632.9±308.2	8637.1±318.5	8794.2±313.4	8678.7±282.2	8578.3±252.8
2hr	8306.5±291.9	8312.5±292.5	8134.9±321.7	8326.4±251.9	8253.0±272.5
3hr	8094.4±301.5	8005.5±300.0	7844.6±294.4	8068.4±276.2	8130.7±288.1
4hr	8293.3±367.5	8044.0±246.6	7930.6±298.8	7838.2±235.7	8294.7±263.6
5hr	8111.8±279.8	8197.9±308.9	8025.3±283.2	7975.6±291.9	8179.4±231.8
Values are mean±SEM.					

Table 6: Rate pressure product data of men and women ingesting theacrine and/or caffeine.

Trail Making Test

Regarding the TMT data, no overall condition effects were noted (all p-values were greater than 0.05). A gender effect was observed for TMT version B, with women performing better than men (p=0.0001). A trend for a gender effect was noted for TMT version A (p=0.09), with women performing better than men. The same was true for the combination of A+B, with women outperforming men (p=0.0007). In addition, a time effect was noted for version A (p=0.0002), with values at 2 and 4 hours post ingestion worse than at pre ($p<0.05$). A time effect was also noted for version B (p=0.01), with values at 2 hours post ingestion better than at pre and 4 hours post ingestion ($p<0.05$). No other overall effects were noted for TMT ($p>0.05$).

Subjective Feelings

With regards to the subjective feelings data, multiple effects were observed, and these are presented in (Table 7), with noted p-values indicated for the respective analyses. The results from our a priori analyses of variables of greatest interest are presented below.

Test	Gender	Condition	Time	GxC	CxT	GxT	GxCxT
Attentive	0.02	0.02	<0.00	0.49	1	0.96	1
Tense	0.37	0.05	0.92	0.09	0.99	0.97	1
Bright	0.16	0.19	0.05	0.34	1	0.57	1
Shaky	0.03	0	0.13	0	0.78	0.92	0.68
Alert	0.3	0.01	0	0.28	1	0.99	1
Groggy	0	<0.00	<0.00	0	1	0.97	0.95
Focused	0.36	0	<0.00	0.18	1	0.99	1
Jittery	0	<0.00	0.09	0	0.86	0.85	0.66
Energetic	0.66	0	<0.00	0.11	1	1	1
Lethargic	0.25	<0.00	<0.00	0.01	0.99	0.87	1
Euphoric	<0.00	0.68	0.14	0.21	1	0.71	1
Depressed	0	0.74	0.9	0.55	1	0.95	1
Motivation to Exercise	<0.00	0.02	0.06	0.63	1	1	1
Well Being	<0.00	0.08	0.64	0.65	1	0.99	1
Energy Crash	<0.00	0.13	0.41	0.14	1	0.62	1
Stress	<0.00	0.07	0.57	0.64	1	0.69	1
Note: G=Gender; C=Condition; T=Time							

• Attentive: Gender, women > men; Condition, theacrine+caffeine > all others; Time, pre and 0.5 hours < all others
• Tense: Condition, theacrine+caffeine > caffeine
• Bright: Time, 5 hours > pre
• Shaky: Gender, women > men; Condition, theacrine+caffeine > placebo, 25mg theacrine, and 125mg theacrine
• Alert: Condition, theacrine+caffeine > 25mg theacrine; Time, pre and 0.5 hours < all others
• Groggy: Gender, women > men; Condition, 25mg theacrine > all others; Time, pre > all others
• Focused: Condition, caffeine and theacrine+caffeine > 25mg theacrine; Time, pre and 0.5 hours < all others
• Jittery: Gender, women > men; Condition, caffeine and theacrine+caffeine > 25mg theacrine
• Energetic: Condition, caffeine and theacrine+caffeine > 25mg theacrine; Time, pre and 0.5 hours < all others
• Lethargic: Condition, 25mg theacrine > all others; Time, pre and 0.5 hours > all others
• Euphoric: Gender, women > men
• Depressed: Gender, women > men
• Motivation to Exercise: Gender, men > women; Condition, caffeine > 25mg theacrine
• Well-Being: Gender, men > women
• Energy Crash: Gender, women > men
• Stress: Gender, women > men

Table 7: Probability values for statistical analyses pertaining to subjective feelings data of men and women ingesting theacrine and/or caffeine.

Pairwise comparisons with Bonferroni adjustment revealed significant within condition changes for four subjective feelings: attentiveness, focused, energetic, and motivation to exercise. With respect to the measure of attentive, individuals reported being more attentive 2 (p=0.017) and 3 hours (p=0.024) post-ingestion of the placebo, relative to baseline. Individuals reported being more attentive 2 (p=0.013) and 3 (p=0.013) hours following the ingestion of caffeine only, relative to baseline. Individuals also reported being more attentive 2 (p=0.000) and 3 (p=0.002) hours following the ingestion of theacrine+caffeine, relative to baseline. Individuals reported being more attentive 3 hours post-ingestion of theacrine 125mg, relative to baseline. No other within condition effects were noted for attentiveness (all p>0.05).

Regarding the measure of focus, individuals that ingested caffeine only reported being more focused 2 (p=0.009) and 3 (p=0.001) hours post-ingestion, relative to baseline. Individuals also reported being more focused 2 (p=0.001) and 3 (p=0.001) hours post-ingestion of theacrine+caffeine, relative to baseline. Individuals that ingested theacrine 125mg reported being more focused 3 hours post ingestion (p=0.016), relative to baseline. Reported focus 3 hours post-ingestion of the placebo trended towards significance (p=0.056). No other within condition effects were noted for reported focus (all p>0.05).

With respect to the measure of energy, individuals reported being more energetic 2 (p=0.005) and 3 (0.001) hours post-ingestion of caffeine only, relative to baseline. Individuals that ingested theacrine+caffeine also reported being more energetic 2 (p=0.001) and 3 (p=0.009) post-ingestion, relative to baseline. Individuals that ingested theacrine 125mg reported being more energetic 3 hours post-ingestion (p=0.001), with data for these individuals 2 hours post-ingestion trending towards significance (p=0.104). Individuals that ingested the placebo reported being more energetic 3 hours post-ingestion (p=0.029), with data for these individuals 2 hours post-ingestion trending towards significance (p=0.134). No other within condition effects were noted for energy (all p>0.05).

About motivation to exercise, individuals reported being more motivated to exercise 2 (p=0.005) and 3 (p=0.018) hours following ingestion of caffeine only, relative to baseline. Individuals that ingested theacrine+caffeine reported feeling more motivated to exercise 2 (p=0.006) and 3 (p=0.011) hours post-ingestion, relative to baseline. No other within condition effects were noted for motivation to workout (all p>0.05).

Discussion

Findings from this study indicate that theacrine, when administered as the branded TeaCrine® alone or in combination with caffeine 1) results in a minimal increase in heart rate and blood pressure, 2) does not impact cognitive performance as assessed by the TMT, and 3) may improve selected measures of subjective feelings. These data pertain to healthy men and women given an

acute dose and then monitored over a five-hour ingestion period, with no food consumption during that time.

Heart Rate and Blood Pressure

As can be seen in (Table 3), theacrine at either dosage resulted in a negligible increase in HR in both men and women. For example, the largest increase of approximately 3 bpm was noted for women between pre and 0.5 hours post ingestion of the 125mg dose. No additional increase was noted for either men or women when the theacrine was combined with caffeine. The increase in HR in women was like that observed with caffeine alone (~2bpm), and in men, far less than that observed for caffeine alone. Based on these data, it can be concluded that theacrine ingestion has very little impact on resting HR.

Tables 4 and 5 present BP data. As was the case for HR, theacrine ingestion at either 25mg or 125mg resulted in only a slight increase in SBP and SBP (~3-5 mm Hg), which is like the response noted for the placebo condition and less than what was noted for caffeine alone. When theacrine was combined with caffeine, the response was very similar to what was observed for caffeine alone. These data indicate that theacrine does not significantly impact SBP or DBP and, when combined with caffeine, does not result in any further increase in BP as compared to caffeine ingestion alone.

Table 6 presents RPP data and as expected based on findings for HR and BP, the results are like what has been already presented for those variables. That is, theacrine alone has little impact on RPP, with values like those of placebo treatment. Moreover, the combination of theacrine+caffeine yields data like caffeine alone.

The findings discussed here are consistent with multiple studies that have reported measures of hemodynamic parameters following acute and repeated (up to 8 weeks) theacrine ingestion/ supplementation in humans [7-9]. Using a randomized, double-blind, placebo-controlled, crossover design, Ziegenfuss et al. indicated that acute doses of 200mg of theacrine did not alter HR, SBP, or DBP over the course of a 3-hour period, relative to the placebo condition [7]. Another study reported no discernable changes in HR, SBP, or DBP following 8 weeks of theacrine supplementation (200mg and 300mg) in 60 healthy adults [8]. Moreover, our most recent work suggests that acute ingestion of theacrine in the form of TheaTrim, a dietary supplement that combines theacrine and caffeine in a proprietary blend, produces negligible alterations in HR, SBP, and DBP over a 4-hour period. For example, HR was 3-5bpm lower in the hours following treatment; SBP and DBP were 1-2mmHg higher in the hours following treatment [9].

When taken together, the data from the present study and the other studies mentioned here indicate that acute theacrine supplementation does not pose any safety risk to individuals with regards to reported hemodynamic variables. This is an important finding considering stimulants used in dietary supplements are often scrutinized for their potential to elevate both heart rate and blood pressure. For instance, high doses of caffeine (400mg) have been shown to significantly elevate both SBP and DBP in healthy humans by as much as 10 mm Hg, relative to baseline and a placebo [12]. Ephedrine, a known vasopressor previously used as ephedra in many dietary supplements, has been shown to significantly elevate heart rate (by as many as 15 bpm), systolic blood pressure (by as much as 14 mm Hg), and diastolic blood pressure (by as much as 7.3 mm Hg) when administered acutely in combination with caffeine [13,14]. Because theacrine has yet to demonstrate similar cardiovascular function altering qualities, it can be deemed safer than such stimulants, at this point.

Trail Making Test

Very little difference was noted between conditions for either version A or B of the TMT. Based on these findings, it can be concluded that 125mg of theacrine, alone or in combination with 150mg caffeine, does not impact cognitive performance as measured by the TMT. This agrees with our previous work [9]. Additional studies involving theacrine are needed to determine the potential cognitive effects of this agent.

Subjective Feelings

In terms of the assessment of subjective feelings, both caffeine and theacrine use resulted in improvements in measures of attentive, focus, and energy primarily within the 2-3-hour time post ingestion. The combination of the two ingredients resulted in improvements during this time for all above mentioned variables, in addition to motivation to exercise. Such findings may have implications for individuals engaged in regular exercise programs and/or for those who require a more energetic feel throughout the day to accomplish certain tasks. The impact of this overall improved mood and perceived energy on cognitive performance and "on the job" performance is presently unknown but may be a focus of future investigations involving theacrine.

Traditionally, individuals use caffeinated beverages of dietary supplements to improve subjective feelings of energy and mood, with the expectation that such effects will occur within 30-60 minutes following ingestion. With theacrine, it appears that the effects take longer to realize (i.e., 2-3 hours post ingestion as compared to 30-60 minutes post ingestion) and are more persistent. It follows that the combination of these two agents may the best approach for individuals seeking a sustained feeling of energy. While we did not observe statistically significant interactions in this work related to subjective measures, this could have been since subjects were tested in a fed state, as compared to prior studies reporting effectiveness for theacrine when subjects were in a fasted state. Knowing that a fasted condition might be more conducive to

theacrine effects, those individuals who partake in some sort of calorie restriction (e.g., alternate day fasting, intermittent fasting) may find that theacrine proves helpful during those times when food intake is minimal.

Conclusion

Theacrine is an emerging dietary ingredient with potential to improve subjective mood. It is well-tolerated by men and women, without noted adverse effects. When used in combination with a relatively low dose of caffeine, it may potentially aid physical and work performance. Of course, additional well-controlled studies are needed to determine this.

Acknowledgments

Funding for this work was provided in part by Compound Solutions, Inc. and the University of Memphis.

Authors' contributions

RJB was responsible for the study design, assistance with data analysis, and manuscript preparation. MB was responsible for coordinating subject recruitment, data collection, data entry, and assistance with manuscript preparation. MLS was responsible for statistical analysis and assistance with manuscript preparation. NJGS was responsible for assistance with manuscript preparation. All authors read and approved of the final manuscript.

Conflict of Interest Statement

RJB has been a Consultant for and/or Principal Investigator on research studies funded by various dietary ingredient and supplement companies. All other authors declare no competing interests.

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