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A randomized double blind study of a green and black tea agent, AssuriTEA®, in healthy male subjects

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Study Design: A double blind, randomized placebo controlled clinical study

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ABSTRACT

Background: Green and black teas have known anti-oxidant and anti-inflammatory properties. The current study was a prospective randomized, double-blind, placebo controlled study of 3 doses of a water-extracted green and black tea agent (AssuriTEA® [AT]).

Methods: Subjects who met inclusion criteria were randomized to one of four groups: 1000, 500, or 250 mg AT or placebo for 28 days. At baseline (BL) and day 28, serum antioxidant capacity, quality of life and safety were assessed.

Results: Of the 55 subjects screened, 43 were considered evaluable. Age was 56.77 ± 2.83 years (mean ± SEM). Evaluable subjects demonstrated improved antioxidant status, as measured by ferric iron reducing antioxidant power at all doses over the placebo ($p < 0.05$). There was significant improvement in cellular antioxidant protection in the 1000 mg AT group versus the placebo ($p=0.032$). Glucose levels were higher in the placebo group versus the 250 mg AT group ($p=0.023$) and there was a significant improvement in systolic blood pressure in the 250 mg AT group at day 28 versus BL ($p=0.048$). In the 1000 mg AT group, there was a significant decrease in systolic blood pressure at day 28 versus BL ($p=0.017$). There was a significant improvement in the vitality category in the 1000 mg AT group ($p=0.029$). Overall, AT was safe and well tolerated with no differences in adverse events or serum chemistries between groups.

Conclusions: Results demonstrate that the administration of AT resulted in improvement of serum antioxidant status, vitality level, blood glucose, and systolic blood pressure in test subjects.

Key Words: Green tea, black tea, *Camellia sinensis*, antioxidant status, blood glucose, systolic blood pressure, and quality of life

BACKGROUND

Camellia sinensis (tea) has been widely studied due to its potent antioxidant and anti-inflammatory properties. All teas originate from the leaves of *Camellia sinensis*, but differences in processing yield tea varieties such as green, black and oolong. Out of these varieties, green and black teas have been most frequently studied *in vitro* and *in vivo*. Large epidemiologic studies have demonstrated links between increased tea consumption and decreased frequency of diseases that have pathologies associated with oxidative stress mechanisms and/or chronic inflammation such as obesity, diabetes mellitus, cardiovascular diseases and cancers including breast, prostate and colon cancers. Prospective rodent and human clinical studies have further examined the various mechanisms of action teas [1-5].

For example, it has been demonstrated that green tea processing results in minimal oxidation of natural tea leaf polyphenols, signifying that green tea contains potent and active phytochemicals such as polyphenols and catechins [6]. Arts et al showed that evaluation of a typical green tea extract demonstrated how, in spite of catechins comprising only 31% of the extract, catechins were responsible for 93% of the tea's anti-oxidant capacity [7]. In black tea, the level of phytochemicals also varies. Black tea differs from green tea in its processing. Black tea is made by drying and crushing *Camellia sinensis* leaves during harvesting. This results in the oxidation of the leaves, which converts catechins into other potent polyphenols such as theaflavins and thearubigens [8, 9]. Arts et al also showed that while non-catechin components (i.e. theaflavins, thearubigins, theanine, quercetin, tannins) composed approximately 92% of a typical black tea extract, they were responsible for 66% of its anti-oxidant capacity [7]. Subsequently, identification of these phytochemicals has helped researchers to investigate various molecular mechanisms of action of tea, including antioxidant capacity, apoptotic activity, and effects on a variety of cell signaling cascades, such as nuclear factor kappa B (NFkB) [10-12].

Based on the known potential of tea, the aim of this clinical study was to assess a novel, patent-pending, water-extracted blend of green and black tea extracts, AssuriTEA® (AT), in a human clinical study, in order to confirm its *in vivo* antioxidant capability and to monitor antioxidant levels, dose-response potential and side effects/toxicities. In this study, AT was administered to healthy male subjects in a randomized, double-blind, placebo controlled study in order to test three doses of the study agent versus a placebo over 28 days. The study also measured safety, tolerability, compliance, and other secondary outcomes. The hypothesis was that AT, a combination of green and black tea extracts, may be beneficial in increasing serum antioxidant levels in healthy male subjects.

MATERIALS AND METHODS

Study design and subjects. This was a prospectively designed, Institutional Review Board (IRB) approved (Sky Lakes Medical Center Institutional Review Board, Klamath Falls, OR), human clinical trial performed in accordance with Good Clinical Practices. Eligible participants included men in good health who were 25-70 years of age, who had no course of newly prescribed medication within two weeks of the first study dose, were able to comply with the study requirements, and were also willing to provide written informed consent. Men were randomized to 1 of 4 groups: placebo (n=11), 250mg AT (n=10), 500mg AT (n=11) or 1000mg

AT (n=11). Men were excluded if they had prior history of renal or hepatic insufficiency, scheduled elective surgery or other procedures requiring general anesthesia during the study, donated blood during the study or within the past month, participated in another research study involving an investigational product in the past month, were using any dietary supplement that might affect anti-oxidant status including vitamin E >400IU, vitamin C >500mg, CoQ-10, lycopene, resveratrol, pycnogenol, or any formulation similarly named "Antioxidant formula," or any other dietary supplement thought to affect antioxidant levels. Men who drank >1 cup of tea or >2 cups of coffee or energy drinks per day or had a history of any smoking within the past 3 months were excluded from the study (Table 1).

Table 1. List of exclusion criteria

Exclusion Criteria
• Known renal or hepatic impairment • Scheduled elective surgery or other procedures requiring general anesthesia during the study • Donation of blood during the study or within the past month • Participation in another research study involving an investigational product in the past month • Hypersensitivity or known allergy to green tea or black tea • Hypersensitivity or known allergy to caffeine • Current use of any dietary supplement containing green or black tea (14 day washout allowed) • Current use of any dietary supplements that may affect anti-oxidant status including: o Vitamin E >400IU, Vitamin C >500mg, CoQ-10, Lycopene, Resveratrol, Pycnogenol, Any formulation similarly named "Antioxidant formula", any other dietary supplement in the opinion of the investigator may affect antioxidant levels (14 day washout allowed) • Current intake of more than 1 cup of green or black tea per day • Current intake of more than 2 cups of coffee or other caffeinated beverage (includes energy drinks, soda, etc.) per day • History of any disease or condition which might compromise the hematopoietic, renal, endocrine, pulmonary, central nervous, cardiovascular, immunological, dermatological, gastrointestinal, urogenital or any other body system • History or presence of gastric ulcer or duodenal ulcer • History of autoimmune disorders e.g. systemic lupus erythematosus, hemolytic anemia • History of psychiatric disorder • Recent history (<2 years) of alcoholism or unlikely to refrain from excessive alcohol consumption during the study period, defined as >2 drinks/day • Current smoking or smoking within the past 3 months • Use of any recreational drugs or a history of drug addiction • Any other significant disease or disorder which, in the opinion of the Investigator, may either put the subject at risk because of participation in the study, or may influence the result of the study, or the subject's ability to participate in the study

Subjects who met eligibility criteria were randomized to one of four study arms, 250mg AT (administered as 2 capsules twice a day (BID) of 62.5mg), 500 mg AT (administered as 2 capsules BID of 125 mg), 1000 mg AT (administered as 2 capsules BID of 250 mg) or placebo (also 2 capsules BID), for a total of 4 capsules per day for all groups. Subjects were instructed to maintain baseline consumption of medications and supplements as reported in the medical history, as well as not change diet and exercise habits throughout the intervention. Evaluable

subjects were defined prospectively as subjects who completed the trial with a compliance of greater than 80%, as measured by pill count.

The primary objective of this study was to determine the effects of 28 days of supplementation of AssuriTEA on antioxidant status in healthy human male subjects. All measures were assessed at each time point, baseline (BL) and endpoint (day 28). A telephone call was performed at week 2 to remind subjects to comply with consumption of the study agent and to assess for any adverse events. At BL and day 28, subjects were asked to avoid food and drink except water for 10 hours prior to the blood draw. For the final visit, subjects were asked to take their last dose of the study agent the day before the scheduled visit, about 18 hours prior to the blood draw. The following variables were evaluated for safety and tolerability of the product: adverse events, clinical laboratory tests (hematology and hepatic function) and vital signs (blood pressure, heart rate, oral temperature, and respiration rate). For blood pressure measurements, average values of 3 consecutive blood pressures were taken and recorded after the subject had rested in the laboratory for at least 5 minutes. Adverse events were graded according to the severity (mild, moderate, severe, or life threatening) and relationship to the study medication (not related, unlikely related, possibly related, probably related, definitely related). Safety monitoring was continuously performed over the 28 day study duration and the participants were interviewed and examined by a study physician at the beginning and end of the study. Adverse events were graded using National Cancer Institute, Common Terminology Criteria for Adverse Events, Version 3.0.

Antioxidant tests

In order to perform a comprehensive assessment of serum antioxidant status and to determine the potential for the serum to protect cells and cellular components from oxidative damage, a panel of three separate antioxidant tests was performed. Each method for antioxidant measurements has its advantages and disadvantages; therefore, the use of more than one method is valuable in assessing antioxidant status [13]. The ferric iron reducing antioxidant power (FRAP) assay is a robust assay and has been widely used to evaluate antioxidant potential of polyphenols. However, the FRAP assay does not measure thiol antioxidants, such as glutathione. As a result, the trolox equivalent antioxidant capacity (TEAC) assay was applied in parallel. The cellular antioxidant protection (CAP-e) assay was used to evaluate the serum levels of antioxidants that are able to provide cellular antioxidant protection. Additionally, two further tests were used to evaluate the levels of oxidative damage to lipids, malondialdehyde (MDA) and DNA (8-hydroxy-2'-deoxyguanosine (8-OHdG). The testing of MDA was performed by a colorimetric assay assessing the reaction of MDA and Thiobarbituric Acid (TBA) under high temperature and acid conditions. 8-OHdG was tested using a commercial ELISA kit (Tregiven #4370-096-K).

Questionnaires

Short form-36 (SF 36)

The SF-36 is a multi-purpose, short-form health survey with thirty-six questions. It yields two summary measures of physical and mental health, which are further broken down into 8-subscale profiles measuring physical functioning, role of physical health, bodily pain, general health, vitality, social functioning, role of emotional health, and mental health. All questions are scored

on a scale from 0 to 100, with 100 representing the highest level of functioning possible. Aggregate scores are compiled as a percentage of the total points possible, using the RAND scoring table. The scores from those questions that address each specific area of functional health status are then averaged together, for a final score within each of the 8 dimensions measured. (e.g. pain, physical functioning etc.) [22].

IPAQ

The International Physical Activity Questionnaires (IPAQ) is a set of 4 questionnaires that assesses activity and generic health questions, reliable as a self-administered questionnaire. The main purpose is to provide an assessment of health data related to physical activity. The IPAQ is scored by both categorical and continuous indicators of physical activity. The continuous indicator is presented as median minutes/week or MET, and the categorical indicator is presented as high, moderate and low [23].

Study Agent

The study agent was a novel, patent-pending, proprietary blend of green and black tea extracts, AssuriTEA (AT, Kemin Foods, L.C., Des Moines, IA, USA). AT contains at least 40% total polyphenols with a minimum of 20% catechins and theaflavins combined. The study agent was an encapsulated dietary supplement which contained 0, 62.5mg, 125 mg or 250 mg of AT. Men were instructed to take 2 capsules twice a day with breakfast and lunch for a total dose of 0 mg (placebo), 250 mg AT, 500 mg AT or 1000 mg AT per day. The study agent was formulated under Good Manufacturing Practices, produced, encapsulated and packaged in 120 capsule quantities into light resistant plastic bottles. The product lots were tested for toxins including heavy metals, pesticides and for excipients. The placebo agent contained an inert substance and was aesthetically matched to the study agent. Stability of the capsules was confirmed throughout the study period (data not shown).

Statistical analyses

Statistics are presented as mean ± SEM for normally distributed variables (e.g., age). Demographic data for the 4 treatment groups were compared with ANOVA. For the primary outcome variables each group was compared to the placebo using a one-tailed t-test based on previous knowledge of the ability of tea to positively influence antioxidant status and all other outcome measures were evaluated using two-tailed t-tests. Where percent change from baseline is reported, the value was calculated for each individual and then averaged to determine the group mean. Statistical significance was defined at $p < 0.05$.

RESULTS

Subjects

Fifty-five subjects were screened, of which 49 were entered into the study and 43 were defined as evaluable upon study completion (Figure 1).

Figure 1: Subject Flow

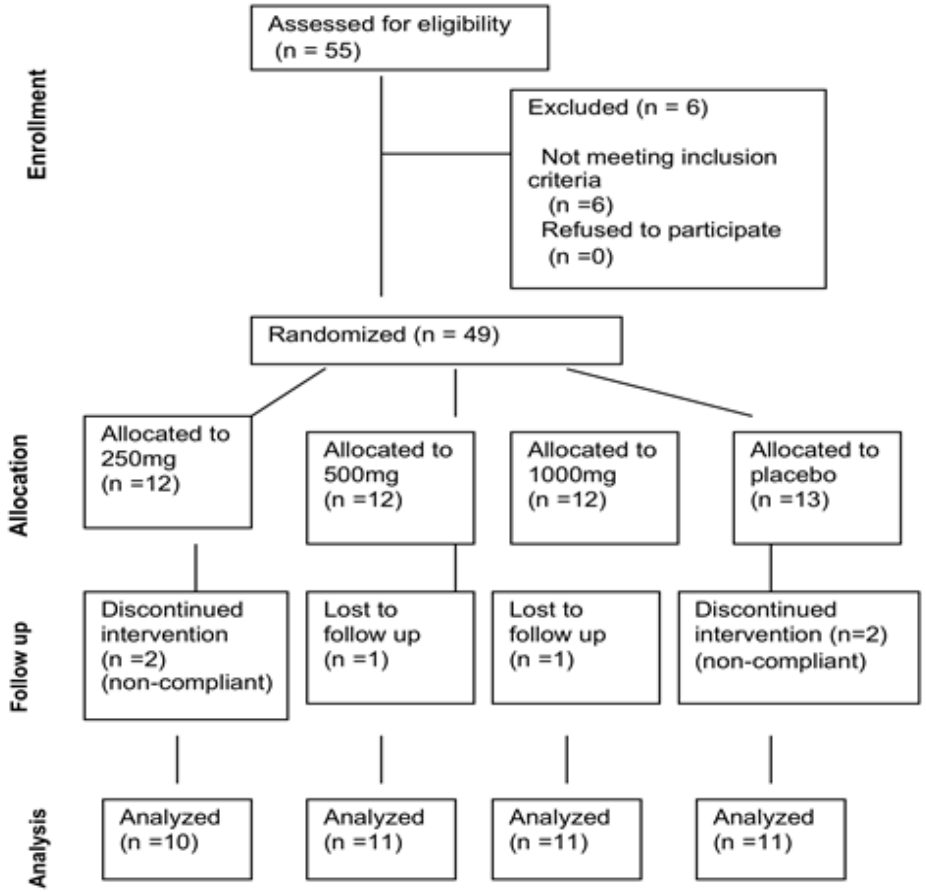

Figure 1. Study flow diagram. This flow diagram shows the subjects enrolled, randomized and allocated to each treatment group, and the subject breakdown within the evaluable group.

The 250 mg AT, 500 mg AT, 1000 mg AT, and placebo groups consisted of 10, 11, 11, and 11 evaluable subjects, respectively. Mean age for evaluable subjects was 56.77 ± 2.83 years (mean ± SEM). Treatment groups were well balanced in demographics (Table 2).

Table 2. Baseline Demographics

Characteristic	Arm 1: 250 mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo	p-value
N	10	11	11	11	n/a
Height (cm)	176.35 (5.77)	178.16 (13.74)	178.31 (6.55)	178.16 (7.75)	0.955
Weight (kg)	89.83 (13.95)	89.44 (10.26)	83.56 (13.28)	96.47 (25.85)	0.363
BMI (kg/ m²)	28.78 (3.15)	28.41 (4.12)	28.45 (4.31)	30.17 (7.08)	0.218
Age (years)	54.10 (2.93)	49.10 (3.72)	49.81 (3.24)	54.27 (4.19)	0.572

Mean (SEM)

Results for the evaluable subjects are described below.

Antioxidant tests

FRAP

The mean percent change from BL to day 28 for the FRAP value increased versus the placebo in the subjects consuming 250, 500 and 1000 mg AT per day (Figure 2).

Figure 2: Ferric Reducing Antioxidant Power

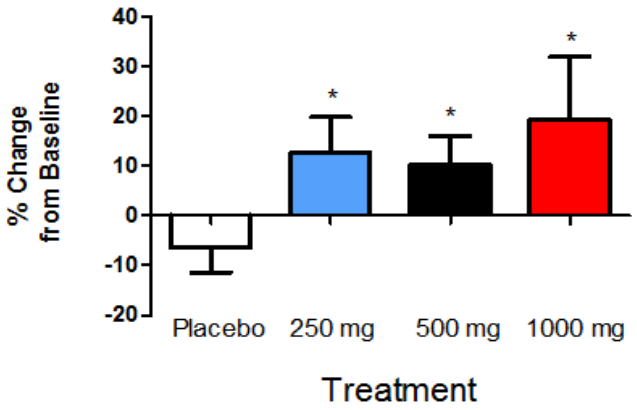

Figure 2. Mean percentage change from baseline in all dose groups. FRAP levels increased in a dose-dependent manner in subjects consuming 250, 500 and 1000 mg AT per day versus placebo after 4 weeks of treatment. *p<0.05 versus placebo.

The mean percent change from BL to day 28 for FRAP values was 12.68 ± 7.20%, 10.42 ± 5.68%, and 19.44 ± 12.61% in the 250, 500 and 1000 mg AT groups respectively vs. placebo -6.32 ± 5.11% (p=0.021, p=0.020, p=0.036, for 250, 500, and 1000 mg AT groups respectively). The raw values are listed in Table 3.

Table 3. Antioxidant Measurements

Measurement	Arm 1: 250 mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo
FRAP (umol/L)				
Day 0	5.93 (0.62)	6.46 (1.10)	6.31 (0.93)	6.29 (0.67)
Day 28	6.81 (1.01)*	7.05 (1.20)*	7.18 (1.13)*	5.72 (0.46)
CAP-e (Fluorescence units)				
Day 0	31191 (2486)	29168 (962)	28919 (1648)	29326 (1432)
Day 28	29705 (2058)	30195 (868)	27823 (1743)*	29933 (901)

Mean (SEM), * P < 0.05 vs. Placebo

CAP-e

The mean percent change from BL for CAP-e decreased (demonstrating improved antioxidant status) at day 28 in the 1000 mg AT dose group versus the placebo (-4.07 ± 1.78% [1000 mg AT], 3.16 ± 3.50% [placebo], p=0.034). (Figure 3, Table 3).

Figure 3: Cellular Antioxidant Protection Assay

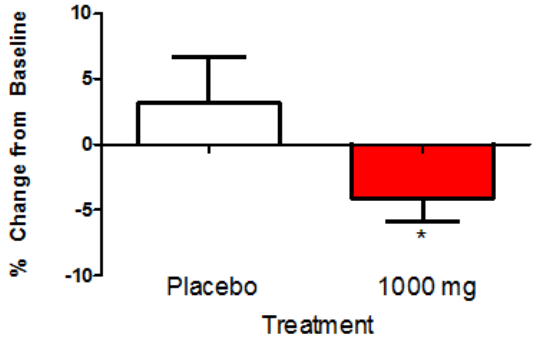

Figure 3. Mean percent change from baseline for CAP-e. Decreased CAP-e (demonstrating improved antioxidant status) was shown at day 28 in the 1000 mg AT dose group versus placebo *p<0.05 versus placebo.

8-OHdG, MDA and TEAC

There were no significant differences in 8-OHdG, MDA or TEAC across the groups.

SF-36

There was a significant difference in the vitality domain of the SF-36 from day 0 to 28 in the 1000 mg AT dose group versus the placebo (Table 4). No other differences were identified.

IPAQ

Table 4. SF 36 Results

Domain	Arm 1: 250mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo
Physical functioning				
Day 0	98.0 (1.1)	98.2 (1.01)	99.1 (0.6)	96.5 (1.5)
Day 28	96.0 (1.6)	97.3 (1.5)	98.6 (0.7)	95.5 (1.7)
Role limitations due to physical health				
Day 0	91.6 (3.9)	77.5 (10.9)	88.6 (7.3)	91.6 (7.9)
Day 28	92.5 (3.8)	90.9 (9.09)	100 (4.8)	95 (3.3)
Role limitations due to emotional problems				
Day 0	93.3 (0.0)	90.9 (6.4)	84.8 (9.4)	93.3 (4.4)
Day 28	100.0 (0.0)	93.9 (6.1)	100 (0.0)	100 (0.0)
Vitality				
Day 0	71.5 (4.4)	74.5 (5.9)	72.3 (6.5)	73 (5.4)
Day 28	78.0 (4.0)	79.1 (6.3)	84.5 (4.0)	70.5 (4.2)*
Emotional well-being				
Day 0	90.4 (1.9)	89.8 (2.8)	92 (2.0)	88 (2.2)
Day 28	89.2 (2.8)	88 (3.9)	93.8 (1.2)	83.2 (5.5)

Social functioning				
Day 0	100 (0.0)	94.3 (3.5)	95.5 (3.4)	98.7 (1.3)
Day 28	98.8 (1.3)	88.6 (6.8)	100 (0.0)	91.3 (5.9)
Bodily Pain				
Day 0	83.5 (4.6)	82.7 (5.6)	82.7 (5.4)	83.3 (5.2)
Day 28	83.5 (5.1)	85.2 (5.2)	90.7 (2.6)	82.3 (4.8)
General Health				
Day 0	87.0 (3.5)	79.1 (5.6)	81.4 (5.8)	80.5 (4.6)
Day 28	86.0 (3.4)	82.7 (5.1)	82.7 (3.1)	79.5 (3.7)

Mean (SEM), * P < 0.05 vs. Placebo

There were no significant differences in either the categorical or MET scores in any of the AT dose groups versus the placebo**.**

Safety and Tolerability

Safety and tolerability were assessed through evaluation of adverse events, clinical laboratory testing, and vital signs. Of the subjects who completed the study, one reported an adverse event, deemed probably relating to the study agent, which included a report of stomach upset and gas (1000 mg AT group). Two additional subjects reported adverse events that were deemed not related to the study agent, with one report of shingles (500 mg AT group) and one report of a laceration on the forearm (placebo group). During the course of the study, 2 subjects were lost to follow up and 4 completing subjects had a compliance of <80%, leaving 43 evaluable subjects. No subjects were discontinued because of any elevated liver function test, clinically significant abnormalities in any other laboratory assessment, or changes in any of the vital sign parameters. Additionally, there was also significant improvement in systolic blood pressure in the 250 mg AT group versus BL (125.90 ± 3.05 vs. 120.10 ± 2.52 mm Hg, day 0 vs. day 28, respectively, p=0.048, [Table 5]).

Table 5. Blood Pressure

Category	Arm 1: 250 mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo
SBP (mm HG)				
Day 0	125.90 (3.05)	124.73 (2.75)	125.11 (2.82)	126.75 (3.32)
Day 28	120.10 (2.52)^	122.73 (2.40)	119.00 (2.15)^	125.83 (1.92)
DBP (mm HG)				
Day 0	76.60 (2.74)	78.09 (2.14)	79.89 (2.34)	78.18 (3.85)
Day 28	74.70 (2.58)	77.45 (3.14)	75.89 (1.80)	76.00 (2.75)

Mean (SEM), ^ P < 0.05 vs. Day 0

In the 1000 mg AT group, there was a significant decrease in systolic blood pressure (125.11 ± 2.82 vs. 119.0 ± 2.15 mm Hg) and a decreasing trend in diastolic (79.89 ± 2.34 vs. 75.89 ±

1.80 mm Hg) blood pressure at day 28 versus day 0 (p=0.017, p= 0.067 systolic, diastolic respectively). There was a significant difference (p=0.023) in fasting serum blood glucose at Day 28 in the 250 mg AT group versus placebo (88.50 ± 5.82 vs. 94.91 ± 6.02 mg/dL, 250 mg AT and placebo, respectively) and a trend toward significance in the 1000 mg AT group versus placebo was observed [Table 6].

Table 6. Blood Glucose

Category	Arm 1: 250 mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo
Serum glucose (mg/dL) Day 0 Day 28	 90.20(8.28) 88.50(5.82)^	 93.09(10.50) 96.36(7.42)	 92.18(8.55) 89.36(6.64)	 93.27(7.35) 94.91(6.02)

Mean (SEM), ^ P < 0.05 vs. Day 0

Laboratory values shown in Table 7. All subjects tolerated the study agent and no test-article related changes in clinical laboratories were identified.

Table 7. Laboratory Values

Category	Arm 1: 250 mg	Arm 2: 500 mg	Arm 3: 1000 mg	Arm 4: Placebo	p-value 250 mg vs. placebo*	p-value 500 mg vs. placebo*	p-value 1000 mg vs. placebo*
WBC (ul) Day 0	5.75(1.01)	5.52 (1.67)	4.97 (1.16)	5.80(1.60)	0.934	0.754	0.188
WBC (ul) Day 28	6.31(1.12)	5.27 (0.51)	5.14 (1.30)	5.52(1.31)	0.164	0.580	0.508
RBC (ul) Day 0	5.08 (0.29)	5.12 (0.34)	4.91 (0.28)	5.04 (0.18)	0.732	0.510	0.204
RBC (ul) Day 28	5.09 (0.25)	5.15 (0.37)	4.83 (0.30)	5.02 (0.20)	0.490	0.338	0.113
HGB (g/dL) D0	16.01 (1.08)	15.76(0.93)	15.60(0.61)	15.75 (0.82)	0.553	0.972	0.639
HGB (g/dL) D28	16.05 (0.96)	15.75(1.0)	15.35 (0.78)	15.56 (0.63)	0.194	0.618	0.498
HCT (%) D0	46.67(3.27)	46.08(2.44)	45.03(2.26)	45.45(1.93)	0.323	0.521	0.651
HCT (%) D28	46.65(2.65)	46.26(3.02)	44.29(2.09)	45.53(1.90)	0.292	0.526	0.173
MCV (fL) D0	91.86(2.79)	90.18(4.69)	91.85(2.14)	90.17(1.51)	0.110	0.994	0.054*
MCV (fL) D28	91.72(2.43)	90.02(4.46)	91.80(2.84)	90.76(2.34)	0.380	0.647	0.374
Platelet (ul) D0	198.50(27.35)	192.09(35.47)	211.82(68.82)	203.90(51.47)	0.773	0.544	0.770
Platelet (ul) D28	214.10(42.30)	174.70(38.74)	217.27(63.15)	180.11(72.95)	0.219	0.838	0.226

Mean (SEM) *p<0.05 ANOVA

DISCUSSION

Many studies have demonstrated that both green and black teas have potent anti-inflammatory and anti-oxidant capabilities. The aim of this study was to analyze three different dosages of a novel green and black tea blend on serum antioxidant parameters and quality of life, and to assess the safety and tolerability of the study agent in healthy human male subjects by evaluating changes in the subjects' blood chemistries and vital signs. A panel of testing is beneficial in the evaluation of the antioxidant capabilities of an ingredient, because antioxidant compounds may operate through multiple mechanisms of action. Moreover, it has been shown that different varieties of tea may have different antioxidant capacities [24].

Our study showed that healthy male subjects demonstrated increased levels of serum FRAP, those who took any of the three doses of AT versus the placebo at day 28. This demonstrates that

the administration of AT is bioavailable, as shown by the results of serum antioxidants levels. Recent studies have also confirmed that both green and black teas are bioavailable and consumption favorably impacts markers of oxidative stress including FRAP [29, 30]. A study examining the antioxidant capacity of green and black tea extracts recently revealed differences in polyphenol content between green tea and black tea extracts respectively [31]. Differences FRAP power in a variety of foods and beverages and specifically between both green and black tea varieties has also been well established [32]. Our study also showed a concurrent percentage change in CAP-e in the 1000 mg AT dose group. CAP-e measures the ability of cells to quench free radicals [25]. Given the approximate 18 hour duration between the last dose of the study agent and the day 28 blood draw, this result demonstrates that a chronic antioxidant effect was achieved with AT supplementation as measured by CAP-e. Taken together, these results demonstrate how AT is a potent antioxidant which can improve antioxidant status.

It is also well known that antioxidant status may be beneficial in the promotion of health and possible prevention of a variety of diseases linked to chronic inflammation including cardiovascular disease, metabolic diseases, such as diabetes mellitus, and many cancers [26-28]. In a study involving young Japanese women, total antioxidant capacity was shown to be inversely related with elevated serum C reactive protein and antioxidant capacity was strongly associated with increased consumption of teas including green, barley and oolong teas [33]. In another study of AT, administration of AT was shown to improve lower urinary tract symptoms in men. The link between inflammatory changes with the prostate and lower urinary tract symptoms has also recently investigated and suggested [34, 35]. Finally, in house *ex vivo* anti-oxidant data was confirmed *in vivo* in the current study at 1000 mg AT daily (data not shown).

Our study also reported significantly lower systolic blood pressure levels in both the 250 mg and 1000 mg AT groups at day 28 versus BL and a trend towards lower diastolic blood pressure in the 1000 mg AT group. A number of studies have demonstrated that green tea may help modulate blood pressure, possibly through mediation of inflammatory pathways such as NFkB [36]. A recent study of green tea in a murine model of vascular inflammation showed that administration of epigallocatechin gallate (a component in both green and black teas) led to a reduction in circulating chemokines and decreases in blood pressure [36]. Another clinical trial of 56 obese subjects with hypertension showed that green tea extract consumption (379 mg daily of green tea extract [including 208 mg of EGCG]), significantly decreased both systolic and diastolic blood pressure by 4 points each after 3 months of supplementation (145 ± 10 at BL versus 141 ± 8 mm Hg at 3 months [p=0.004], and 88 ± 4 versus 84 ± 3 mm Hg [p <0.001], systolic and diastolic, respectively) [37-32]. Administration of black tea has also been shown to increase pulse wave velocity, decrease augmentation index, and increase flow-mediated dilation in healthy subjects [38, 39, 33, and 34].

Our study also confirmed additional benefits for subjects who consumed AT for 28 days. These measures included improved fasting glucose levels and increased energy, as measured by a quantitative questionnaire. In our study, subjects in the 250 mg and 1000 mg AT groups versus the placebo reported significantly lower fasting serum blood glucose levels after administration of the study agent for only 28 days. The benefit of improved serum glucose was detected sooner in comparison to another study by Bogdanski et al, which also demonstrated improved serum fasting blood glucose levels in subjects treated with green tea extract versus controls after 3

months (5.5 ± 0.4 vs 5.0 ± 0.3 mmol/L [p=0.016], BL versus 3 months, respectively) [37, 33]. The relationship between green tea, hypertension and insulin sensitivity is currently unclear and unknown. However, hypotheses for these relationships include the role of hyperinsulinemia and sympathetic nervous system activation that may also be related to obesity related causes of hypertension, especially in regard to the renin-angiotensin-aldosterone system in regulating blood pressure [40]. Finally, significant improvements in energy were shown in our study, with subjects who consumed 1000mg of AT demonstrating significantly improved scores in the vitality subscale of the SF-36 questionnaire. To our knowledge, this is the first study showing self-reported increases in vitality as a result of supplementation with a blend of green and black tea.

Limitations of the current study include the short intervention period. However, the results obtained after only 28 days of supplementation indicate the potency of the study agent and warrant further long-term analysis. Strengths of the current study include its multi-dose, randomized, double-blind, placebo controlled design and evaluation of antioxidant status using several assays.

CONCLUSION

In conclusion, this study demonstrated that 28 day supplementation with AT improved serum antioxidant status, systolic blood pressure, glucose and self-reported energy/vitality. The administration of AT up to 1000 mg daily was also safe and well tolerated.

List of abbreviations:

AT AssuriTEA®;
BID Twice a day;
BL Baseline;
CAP-e Cellular antioxidant protection;
EGCG Epigallocatechin gallate;
FRAP Trolox equivalent antioxidant capacity;
IPAQ International physical activity questionnaire;
IRB Institutional Review Board;
LUTS Lower urinary tract symptoms;
MDA Malondialdehyde;
SEM Standard error of the mean;
SF-36 Short form 36;
TEAC Trolox equivalent antioxidant capacity

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Competing interests: Kelli Herrlinger-Author was employed by the sponsor company and manufacturer of the ingredient AssuriTEA at the time of the clinical trial. However, a third party study site conducted the trial on behalf of the sponsoring company. Diana Chirouzes-Author was employed by the sponsor company and manufacturer of the ingredient AssuriTEA at the time of the clinical trial. However, a third party study site conducted the trial on behalf of the sponsoring company. Michael Ceddia- Author was employed by the sponsor company and manufacturer of the ingredient AssuriTEA at the time of the clinical trial. However, a third party study site conducted the trial on behalf of the sponsoring company. Jillian Capodice- Author has consulted for the sponsor company. Aaron Katz- Author has consulted for the sponsor company

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