Sponsor: **A. Menarini Industrie Farmaceutiche Riunite S.r.l.** **Protocol Version n° 0.0 – *09 October 2017***

Study Code MEIF/17/BAC-COG/001

CLINICAL STUDY PROTOCOL

Title: "Effect of an antioxidants mix on cognitive performance and well being: The Bacopa, Licopene, Astaxantina, Vitamin B12" (Acronym BLAtwelve study)

STUDY CODE: MEIF/17/BAC-COG/001

Study type and design: randomized, double-blind, placebo-controlled, parallel-arm, superiority study

Phase: not applicable (food supplement study)

Protocol Version n° 0.0 Emission Date: 09/10/2017

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1. RESPONSIBILITIES

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2. PROTOCOL SYNOPSIS

Title Acronym Study Code	Effect of an antioxidants mix on cognitive performance and well-being: The Bacopa, Licopene, Astaxantin, Vitamin B12 BLAtwelve study MEIF/17/BAC-COG/001
Investigational Product Reference Therapy (comparator) Dose Regimen	A mix of four bioactive compounds: bacopa, lycopene, astaxanthin, vitamin B12. Placebo. Once daily.
Study Type and Design	9-weeks double-blind, randomized, placebo-controlled, parallel-arm superiority study.
Phase	Not applicable.
Background Rationale	Long-term oxidative stress is believed to represent one of the most important factor contributing to the decline of cognitive function often observable with aging. Oxidative stress, due to the generation of free radical resulting from normal metabolism, is usually maintained at low level by antioxidant system. However, in some conditions oxidant/antioxidant balance can be perturbed by increased generation of reactive oxygen species and/or decreased endogenous ability to counteract them [Praticò et al., 2008]. Brain tissue is highly sensitive to oxidative stress because it has a high request for oxygen and has a relative weakness of antioxidant systems. Furthermore, brain also contains high levels of polyunsaturated fatty acid, making it more vulnerable to oxidative injuries [Praticò et al., 2008]. Altered mitochondrial function, the amyloid β peptides and the presence of unbound trace metal ions represent the most investigated potential sources of oxidative stress in the brain [Reddhy et al., 2005; Mattson MP, 2004]. Depending on the biomolecules attacked by reactive oxygen species, oxidative stress can promote peroxidation of protein, lipid and nucleic acids thus favouring the onset and progression of cognitive dysfunction during aging [Praticò D et al., 2008]. During the last few years an increasing interest has been focused on antioxidants such as carotenoids, flavonoids and vitamins as potentially useful agents in the prevention of the onset and progression of cognitive dysfunction [Rao et al., 2013; de Rijk et al., 1997; Deschamps et al., 2001; Engelhart et al., 2002]. In this regard, lycopene, a lipid-soluble carotenoid compound, well represented in tomatoes and red fruits, including watermelon, pink grapefruit and guava, has been proposed as a brain protective agent. In vitro studies indicated that lycopene protects against neuronal death induced by different neurotoxic compounds, including 1-methyl-4-phenylpyridinium (MPP+), methylmercury, amyloid β, trimethyltin and 6-

	hydroxydopamine [Di Matteo et al., 2009; Yi et al.;2013]. Furthermore, animal experiments using rat models demonstrated that lycopene prevents brain injury caused by focal or global ischemia and reperfusion [Fujita et al., 2013] and alleviates cognition dysfunction induced by colchicine and rotenone [Kaur et al., 2011]. A population-based follow-up study demonstrated that males in the highest quartile of serum lycopene concentrations exhibited 59 and 55% lower risks of ischemic stroke when compared with males in the lowest quartile [Karppi et al., 2012]. Astaxanthin is a xanthophyll carotenoid nutrient known for having potent antioxidant and anti-inflammatory actions thanks to molecular properties that precisely position it within cell membranes and circulating lipoproteins [Kidd, 2011; Lim SY et al. 2016]. Astaxanthin has shown a variety of brain benefits under experimental conditions [Kidd, 2011; Lim SY et al. 2016]. Bacopa monniera is a creeping herb extensively investigated for its pharmacological and therapeutic effects. Its ethanol extract contains a mixture of triterpenoid saponins designated as bacosides A and B [Chatterjee et al., 1963; Chatterjee et al., 1965]. In vitro studies using Bacopa Monnieri have shown that it inhibits free radical formation and DNA damage in a dose dependent manner [Russo et al.;2003]. Promising indications for use in humans include improving cognition in the elderly and in patients with neurodegenerative disorders [Stough C et al., 2013]. Vitamin B12, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and for the formation of red blood cells [Aisen et al.; 2008]. Serum levels in the subclinical low-normal range (<250 pmol/L) are associated with Alzheimer's disease, vascular dementia and Parkinson's disease [Moore et al.; 2012] while some evidences suggest that vitamin B12 administration might be useful in preserving brain health. Starting from these evidences it is conceivable that a food supplement containing bacopa, lycopene, astaxantina, vitamin B12 could be effective in improving brain health.
Aim of the Study	To evaluate the influence of a mix of four bioactive compounds – bacopa, lycopene, astaxanthin and vitamin B12 – on cognitive performance, mood state and well-being in subjects aged ≥ 60 years with no evidence of cognitive dysfunction.
Primary Objective	Evaluation of changes in Trail Making Test (TMT) scores from baseline (V2) to 8 weeks of treatment (V4), analyzed in the following hierarchical order: TMT-B, TMT-A and TMT B-A.
Secondary Objectives	1. Change of Verbal Fluency Test (VFT) score from baseline (V2) to 8 weeks of treatment (V4). 2. Changes of Montreal Cognitive Assessment (MoCA) score from baseline (V2) to 8 weeks of treatment (V4). 3. Changes of Mini Mental State Examination (MMSE) score from

	baseline (V2) to 8 weeks of treatment (V4). 4. Changes of Rey Auditory Verbal Learning Test (AVLT) from baseline (V2) to 8 weeks of treatment (V4). 5. Changes of psychological well-being as assessed by General Health Questionnaire (GHQ-12) from baseline (V2) to 8 weeks of treatment (V4). 6. Changes of mood states as assessed by the Profile of Mood Stated (POMS) from baseline (V2) to 8 weeks of treatment (V4). 7. Changes of sexual satisfaction as evaluated by the New Sexual Satisfaction Scale (NSSS) from baseline (V2) to 8 weeks of treatment (V4). 8. Changes of metabolic parameters from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4): A. glucose B. insulin C. Homeostatic Model Assessment - Insulin Resistance (HOMA-IR) derived from glucose and insulin D. total cholesterol E. Low Density Lipoprotein (LDL) cholesterol F. High Density Lipoprotein (HDL) cholesterol G. triglycerides H. uric acid 9. Changes of plasma markers of oxidative stress from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4): A. 8-iso-Prostaglandin F2alpha B. Plasma malondialdehyde
Subjects characteristics Number of Subjects	Cognitively-intact individuals aged 60 years or more. No. 80 (including a possible 15% for screening failure and drop-out) participants with a ratio of 1:1 for the 2 treatment groups.
Number of Centers & Countries	1 center, Geriatric Division, P.O. SS Filippo e Nicola, Avezzano, Italy
Study Duration (specify different study phases) First Pts In (FPI) Last Pts Last Visit (LPLV):	4 months January 2018 April 2018

Study Procedures	Cognitive functions, mood state, self-perceived well being and sexual satisfaction will be assessed at baseline and after 8 weeks of regular consumption of the mix of the four antioxidant compounds. Furthermore, blood pressure, metabolic variables and plasma markers of oxidative stress will be assessed at baseline, after 4 and after 8 weeks of regular consumption of the antioxidant compounds. **A. COGNITIVE PERFORMACES WILL BE EVALUATED BY TMT, VFT, MOCA, MMSE AND AVLT:** TMT, which explores visual-conceptual and visual-motor tracking, is a frequently used neuropsychological test because of its sensitivity to brain damage. TMT is administered in two parts. Part A is a visual-scanning, timed task where participants are asked to connect with lines 25 circles numbered from 1 to 25 as quickly as possible. The test is terminated after 5 minutes even if not completed. In Part B participants are asked to connect circles containing numbers (from 1 to 13) or letters (from A to L) in an alternate numeric/alphabetical order. The test is terminated in every case after 10 minutes even if not completed. The TMT B-A score calculated as the difference between TMT-B and TMT-A times is considered a measure of cognitive flexibility relatively independent of manual dexterity. VFT is a short test of verbal functioning. Participants are given 1 min to produce as many unique words as possible within a semantic category (category fluency) or starting with a given letter (letter fluency). The participant's score in each task is the number of unique correct words. MoCA assesses a broader array of cognitive domains (e.g., attention/executive functioning, visuospatial abilities and language) and it has been demonstrated to be able to detect cognitive impairment with scores ranging from 0 to 30. MMSE is a widely used screening tool for cognitive impairment and covers five areas of cognitive function including orientation, attention, calculus, recall and language with scores ranging from 0 to 30. AVLT is a neuropsychological assessment designed to evaluate the nature and severity of memory dysfunction and to track changes in memory function. The examiner reads aloud a list of 15 words at the rate of one per second. The participant is then asked to repeat all words from the list that she/he can remember. This procedure is carried out a total of five times. After a 15-minute delay, the participant is again asked to recall as many words as possible from the first list. The participant is then requested to read a list of words and asked to indicate whether each word was from the first list. The score for each trial is the number of words correctly recollected. **B. MOOD STATES WILL BE ASSESSED BY POMS:** POMS is a widely used tool in assessing mood states that has already

	been validated for Italy. Higher scores reflect mood decrements, except for the vigor subscale, where higher scores reflect improved mood. Taking into consideration the two components characteristic of the POMS that measure negative affect and positive affect, respectively, the data collected with the questionnaire will be analyzed both as global score and by separately considering a Positive Affect Scale (PAS-POMS, 48 items, score ranging from 0 to 192) and a Negative Affect Scale (NAS-POMS, 10 items, score ranging from 0 to 40). **C. PSYCHOLOGICAL WELL-BEING WILL BE ASSESSED BY GHQ-12:** GHQ-12 is a relevant instrument for measuring psychological well-being. It has been extensively evaluated in terms of validity and reliability as a one dimensional indicator of the severity of psychological morbidity, and it has already been validated for Italy. **D. SEXUAL SATISFACTION WILL BE EVALUATED BY THE NSSS:** NSSS is a 20 item, multidimensional, composite measure of sexual satisfaction based on a five-dimension, conceptual model that emphasized the importance of multiple domains of sexual behavior including sexual sensations, sexual awareness and focus, sexual exchange, emotional closeness, and sexual activity. **E. BLOOD PRESSURE, METABOLIC VARIABLES, AND PLASMA MARKERS OF OXIDATIVE STRESS WILL BE ASSESSED AT BASELINE, AFTER 4 AND 8 WEEKS OF REGULAR CONSUMPTION OF THE ANTIOXIDANT COMPOUNDS:** - clinic systolic and diastolic blood pressure levels will be recorded in the morning, using a validated oscillometric device with appropriately sized cuffs on the non-dominant upper arm after 5 min resting in a seated position; the first blood pressure measurement will be discarded and the subsequent three consecutive blood pressure readings, taken at 3-min intervals, will be recorded. The average of these latter measures will be considered for statistical analysis. [O'Brien et al., 2005]. - Metabolic profile will be evaluated by the following variables: A. glucose (normal range 60-100 mg/dL) B. insulin (normal range 2.7-10.4 mcUI/mL) C. HOMA-IR (derived from glucose and insulin) D. total cholesterol (normal range 150-200 mg/dL) E. LDL cholesterol (normal range 40-130 mg/dL) F. HDL cholesterol (normal range 35-90 mg/dL) G. triglycerides (normal range 60-170 mg/dL) H. uric acid (normal range 3.5 – 7.0 mg/dL) - Oxidative stress will be evaluated by measuring circulating levels of 2

	soluble biomarkers: A. 8-iso-Prostaglandin F2alpha (no predefined normal range) B. Plasma malondialdehyde (no predefined normal range) The study will last approximately two months for each subject. A total of 4 study visits will be performed as follows: **VISIT 1 (screening, day -7 to Visit 2):** Subjects who provide written informed consent will undergo screening assessments to evaluate eligibility for the study. Subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for the study and will undergo the following evaluations: 1) Collection of medical history and information about eventual concomitant medications 2) Complete physical evaluation including a) anthropometric parameters (height, weight, waist circumference), b) cardiovascular system, c) respiratory system, d) gastrointestinal system and e) respiratory system 3) Blood pressure measurement Subjects eligible for the study will meet with a dietician in order to evaluate current diet habits and correct any nutritional insufficiencies. Participants will be then instructed to maintain their usual lifestyle and intake of fruits and vegetables and to avoid any food supplement. **VISIT 2 (randomization/baseline, 7 days after V1):** Subjects eligible for the study will undergo the following evaluations: 1) Collection of information about eventual concomitant medications 2) Complete physical evaluation including a) anthropometric parameters (weight, waist circumference), b) cardiovascular system, c) respiratory system, d) gastrointestinal system and e) respiratory system 3) Blood pressure measurement 4) Neuropsychological evaluation (TMT, VFT, MoCA, MMSE, AVLT) 5) Evaluation of mood state (POMS) 6) Evaluation of quality of life (GHQ12) 7) Evaluation of sexual satisfaction (NSSS) 8) Blood samples to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid) 9) Blood samples to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde) Subjects will be randomly assigned to two parallel groups in a 1:1 ratio and treated with a mix of four bioactive compounds: bacopa, lycopene,

	astaxanthin and vitamin B12, (1025,00 mg/daily) or placebo (1025,00 mg/daily, for 2 months. During Visit 2 one month of treatment of IMP (45 tablets) will be given to each subject. **<u>VISIT 3 (Week 4, treatment period, 30 days ± 3 days after V2):</u>** Subjects will undergo to the following evaluations: 1) Collection of information about eventual concomitant medications 2) Complete physical evaluation including a) anthropometric parameters (weight, waist circumference), b) cardiovascular system, c) respiratory system, d) gastrointestinal system and e) respiratory system 3) Blood pressure measurement 4) Blood samples to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid) 5) Blood samples to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde) Eventual adverse events will be checked and evaluated. IMP return/compliance check of first month of therapy. During Visit 3 another one month of treatment of IMP (45 tablets) will be given to each subject. **<u>VISIT 4 (Week 8, final visit, 30 days ± 3 days after V3):</u>** Subjects will undergo to the following evaluations: 1) Collection of information about eventual concomitant medications 2) Complete physical evaluation including a) anthropometric parameters (weight, waist circumference), b) cardiovascular system, c) respiratory system, d) gastrointestinal system and e) respiratory system 3) Blood pressure measurement 4) Neuropsychological evaluation (TMT, VFT, MoCA, MMSE, AVLT) 5) Evaluation of mood state (POMS) 6) Evaluation of quality of life (GHQ12) 7) Evaluation of sexual satisfaction (NSSS) 8) Blood samples to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid) 9) Blood samples to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde) Eventual adverse events will be checked and evaluated.

	IMP return/compliance check.
Inclusion Criteria	1) Subjects aged ≥60 years. 2) Subjects who provide written Informed Consent to the study.
Exclusion Criteria	1) Subjects with cognitive dysfunctions or clinically significant coexisting medical conditions (cardiovascular disease, cerebrovascular events, overt dementia defined by MMSE <27 or other neurological disorders, thyroid disorders, or inflammatory diseases) 2) Subjects with a score on the Geriatric Depression Scale (GDS) >11 in order to avoid confounding due to the influence of concomitant depression on the performance on cognitive tests 3) Current smokers 4) Habitual users of antioxidant supplements (including vitamins C and E) 5) Habitual consumers of chocolate or other cocoa products (daily consumption of any amount) 6) Subjects under treatments with medications known to have antioxidant properties (including statins and glitazones) or to interfere with cognitive functions (including benzodiazepines and antidepressants) 7) Subjects with hypersensitivity to any component of the study medications 8) Subjects who are participating in or having participated in another clinical trial within the previous three months.
Efficacy Evaluation	• *Primary Efficacy Endpoint:* changes of TMT scores from baseline (V2) to 8 weeks of treatment (V4). Changes will be assessed according to the following hierarchical order: TMT-B, TMT-A, TMT B-A. • *Secondary Efficacy Endpoints:* a) changes of VFT score from baseline (V2) to 8 weeks of treatment (V4); b) changes of MoCA score from baseline (V2) to 8 weeks of treatment (V4); c) changes of MMSE score from baseline (V2) to 8 weeks of treatment (V4); d) changes of AVLT score baseline from baseline (V2) to 8 weeks of treatment (V4); d) changes of GHQ-12 score baseline from baseline (V2) to 8 weeks of treatment (V4); e) changes of POMS score from baseline (V2) to 8 weeks of treatment (V4);

	f) changes of NSSS score from baseline (V2) to 8 weeks of treatment (V4); g) changes of metabolic parameters (glucose, insulin, HOMA-IR , total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and uric acid from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4); h) changes of circulating levels of 8-iso-Prostaglandin F2alpha and malondialdehyde from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4). • *Exploratory analyses:* a) correlation between changes of cognitive scores (TMT, VFT, MoCA, MMSE, AVLT) and changes of blood pressure and metabolic parameters including indices of oxidative stress; b) assessment of the effect of time in the change for the primary endpoint.
Safety Evaluation	All adverse events will be duly assessed for identifying any emergent safety finding.
Statistical Assumptions	• Sample size calculation The study will be based on an estimated sample size of 68 subjects, with a ratio of 1:1 for the 2 treatment groups, which has been calculated to be adequate to achieve 90% power to detect a large effect size (f=0.40) using an ANCOVA model with a baseline covariate and an α of 0.05 between treatment and control (G*power version 3.1.9.2). To consider a possible 15% dropout rate, 80 subjects will be enrolled. The three primary variables (changes in TMT-B, TMT-A and TMT B-A scores) will be analyzed in a hierarchical order and no adjustment for sample size is needed. All randomized subjects receiving at least a treatment dose and having the post-randomization efficacy evaluation will be included in the ITT population for efficacy analysis. All randomized subjects without protocol deviations affecting the primary endpoint will be included in the Per-Protocol (PP) analysis. All randomized subjects receiving at least a treatment dose will be included in the safety analysis. • Efficacy analyses The primary variables (changes of TMT-B, TMT-A and TMT B-A scores), will be analyzed in a hierarchical order using an ANCOVA model with the treatment group (main effect) and baseline as covariates. All the other continuous variables (changes of test scores, changes of metabolic parameters, changes of circulating levels of 8-iso-Prostaglandin F2alpha and malondialdehyde) will be analyzed by using an ANCOVA model with the treatment group (main effect) and baseline as covariates. As additional covariate for testing the homogeneity of the regression coefficients, the interaction term of *treatment*baseline* will be included in the model and removed if not significant.

	Categorical variables (gender, eventually present cardiovascular risk factors or pharmacological treatment) will be tested by using a Chi-Square test or the Fisher exact test if necessary. • Exploratory analyses Pearson correlation will be used to evaluate correlations between changes of cognitive scores (TMT, VFT, MoCA, MMSE, AVLT) and changes of blood pressure and metabolic parameters including indices of oxidative stress. Spearman nonparametric correlation will be also applied when one or both of the variables are not assumed to be normally distributed and interval (but are assumed to be ordinal). In order to evaluate and quantify the effect of time itself (i.e. 8 weeks after the treatment intake) in the change for the primary endpoint a two-way analysis of variance (treatment*time) with time as a repeated measure will be also implemented. • Safety analysis Adverse events will be summarized and compared between the two treatment groups. Study product tolerability will be investigated and compared between treatment groups.

2.1 Study Scheme:

Treatment A ---------- Follow Up ---------- Final Visit

Screening ----------

Treatment B ---------- Follow Up ---------- Final Visit

V1----------V2----------V3----------V4

(day -7 to Visit 2) (7 days after V1) (30 days ± 3 days after V2) (30 days ± 3 days after V3)

2.2 Study Flow Chart

	Day -7 to 1	Day 1	Week 4 (± 3 days)	Week 8 (± 3 days) Last visit
	Visit 1	Visit 2	Visit 3	Visit 4
	Screening	Randomization	Treatment	Treatment
Inclusion/exclusion criteria	✓			
Informed consent	✓			
Randomization		✓		
Medical history	✓			
Concomitant medications	✓	✓	✓	✓
Physical examination	✓	✓	✓	✓
Dietitian visit	✓			
Blood pressure	✓	✓	✓	✓
MMSE, GDS	✓			
Neuropsychological evaluation (TMT, VFT, MoCA, MMSE, AVLT)		✓		✓
Evaluation of mood state (POMS)		✓		✓
Evaluation of quality of life (GHQ-12)		✓		✓
Evaluation of sexual satisfaction (NSSS)		✓		✓
Blood samples to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid)		✓	✓	✓
Blood samples to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde)		✓	✓	✓
IMP dispense treatment		✓	✓	
IMP return/compliance check			✓	✓
Adverse events		✓	✓	✓

NOTE:

a. Blood pressure will be taken with the subject in the sitting position (subject seated for 5 minutes). Heart rate will be taken in a resting state (5 minutes).
b. Blood samples will be taken after an overnight fasting
c. Neuropsychological testing will be performed in a quiet room before taking the daily dose of the tested food supplement

3. ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
AVLT	Rey Auditory Verbal Learning Test
CA	Competent Authority
CRA	Clinical Research Associate
CRF	Case Report Forms
CRO	Contract Research Organisation
DCF	Data Clarification Form
DNA	DeoxyriboNucleic Acid
GCP	Good Clinical Practice
GDS	Geriatric Depression Scale
GHQ-12	General Health Questionnaire 12
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
IEC	Independent Ethics Committee
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
ICH-GCP	International Conference of Harmonisation - Good Clinical Practice
ITT	Intent to Treat
LDL	Low-density-lipoprotein
HDL	High-density-lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
MMSE	Mini Mental State Examination
MoCA	Montreal Cognitive Assessment
NSAE	Non-Serious Adverse Event
NSSS	New Sexual Satisfaction Scale
POMS	Profile Of Mood Stated
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SOC	System/Organ Class
TMF	Trial Master File
TMT	Trail Making Test
VFT	Verbal Fluency Test

4. BACKGROUND INFORMATION

Long-term oxidative stress is believed one of the most important factor contributing to the decline of cognitive function often observable with aging. Oxidative stress, due to the generation of free radical resulting from normal metabolism, is usually maintained at low level by antioxidant system. However, in some conditions oxidant/antioxidant balance can be perturbed by increased generation of reactive oxygen species and/or decreased endogenous ability to counteract them [Praticò et al., 2008].

Brain tissue is highly sensitive to oxidative stress because it has a high request for oxygen and has a relative weakness of antioxidant systems. Furthermore, brain also contains high levels of polyunsaturated fatty acid, making it more vulnerable to oxidative injuries [Praticò et al., 2008]. Altered mitochondrial function, the amyloid β peptides and the presence of unbound trace metal ions represent the most investigated potential sources of oxidative stress in the brain [Reddhy et al., 2005; Mattson MP, 2004]. Depending on the biomolecules attacked by reactive oxygen species, oxidative stress can promote peroxidation of protein, lipid and nucleic acids thus favouring the onset and progression of cognitive dysfunction during aging [Praticò D et al., 2008].

During the last few years an increasing interest has been focused on antioxidants such as carotenoids, flavonoids and vitamins as potentially useful agents in the prevention of the onset and progression of cognitive dysfunction [Rao et al., 2013; de Rijk et al., 1997; Deschamps et al., 2001; Engelhart et al., 2002].

In this regard, lycopene, a lipid-soluble carotenoid compound, well represented in tomatoes and red fruits, including watermelon, pink grapefruit and guava, has been proposed as a brain protective agent. In vitro studies indicated that lycopene protects against neuronal death induced by different neurotoxic compounds, including 1-methyl-4-phenylpyridinium (MPP+), methylmercury, amyloid β, trimethyltin and 6-hydroxydopamine [Di Matteo et al., 2009; Yi et al.;2013]. Furthermore, animal experiments using rat models demonstrated that lycopene prevents brain injury caused by focal or global ischemia and reperfusion [Fujita et al., 2013] and alleviates cognition dysfunction induced by colchicine and rotenone [Kaur et al., 2011]. A population-based follow-up study demonstrated that males in the highest quartile of serum lycopene concentrations exhibited 59 and 55% lower risks of ischemic stroke when compared with males in the lowest quartile [Karppi et al., 2012].

Astaxanthin is a xanthophyll carotenoid nutrient known for having potent antioxidant and anti-inflammatory actions thanks to molecular properties that precisely position it within cell membranes and circulating lipoproteins [Kidd, 2011; Lim SY et al. 2016]. Astaxanthin

has shown a variety of brain benefits under experimental conditions [Kidd, 2011; Lim SY et al. 2016].

Bacopa monniera is a creeping herb extensively investigated for its pharmacological and therapeutic effects. Its ethanol extract contains a mixture of triterpenoid saponins designated as bacosides A and B [Chatterjee et al., 1963; Chatterjee et al., 1965]. In vitro studies using Bacopa Monnieri have shown that it inhibits free radical formation and DNA damage in a dose dependent manner [Russo et al.;2003]. Promising indications for use in humans include improving cognition in the elderly and in subjects with neurodegenerative disorders [Stough C et al., 2013].

Vitamin B12, also called cobalamin, is a water-soluble vitamin that has a key role in the normal functioning of the brain and nervous system, and for the formation of red blood cells [Aisen et al.; 2008]. Serum levels in the subclinical low-normal range (<250 pmol/L) are associated with Alzheimer's disease, vascular dementia and Parkinson's disease [Moore et al.; 2012] while some evidences suggest that vitamin B12 administration might be useful in preserving brain health.

Starting from these evidences it is conceivable that a food supplement containing bacopa, lycopene, astaxantina, vitamin B12 could be effective in improving brain health.
The purpose of this study is to evaluate if the mix of these four bioactive compounds, orally administered for 8 consecutive weeks, can be of help on cognitive performance, mood state and well-being in a target population with no evidence of cognitive dysfunctions.
The study will be conducted in compliance with this protocol, GCP and the applicable regulatory requirements.

5. TRIAL OBJECTIVES AND PURPOSE

The aim of this study is to evaluate the influence of a mix of four bioactive compounds – bacopa, lycopene, astaxanthin and vitamin B12 – on cognitive performance, mood state and well-being in subjects aged ≥ 60 years with no evidence of cognitive dysfunction.
The primary objective of the study is to evaluate the changes in TMT scores from baseline (V2) to 8 weeks of treatment (V4), analyzed in the following hierarchical order: TMT-B, TMT-A and TMT B-A
Secondary objectives of this study are to evaluate changes from baseline (V2) to 8 weeks of treatment (V4) in Verbal Fluency Test (VFT) score, Montreal Cognitive Assessment (MoCA) score, Mini Mental State Examination (MMSE) score, Rey Auditory Verbal Learning Test (AVLT), psychological well-being as assessed by General Health

Questionnaire (GHQ-12), mood states as assessed by the Profile of Mood Stated (POMS), sexual satisfaction as evaluated by the New Sexual Satisfaction Scale (NSSS).
Changes of metabolic parameters from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4) will be also evaluated as secondary objectives (glucose, insulin, HOMA-IR, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid).
Changes of plasma markers of oxidative stress from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4) will be evaluated as secondary objectives (8-iso-Prostaglandin F2alpha, Plasma malondialdehyde).
Finally the safety and tolerability of the study product will be assessed.

6. STUDY DESIGN

This study has been designed as 9 weeks double-blind, randomized, placebo-controlled, parallel-arm, superiority study.
The study will be conducted in 1 Italian clinical site and will involve about 80 subjects.

Subjects will be randomly allocated to one of the following groups:

- Group I: mix of the four bioactive compounds (bacopa, lycopene, astaxanthin and vitamin B12), once a day for 8 weeks per os;
- Group II: placebo, once a day for 8 weeks per os.

The study will be double blind. Neither the study staff at clinical sites (Investigators, nurses, pharmacist) nor the subject will be aware of the treatment assigned.
Each participant will attend 4 visits over a total period of about 9 weeks.

6.1 RANDOMIZATION

Each subject for whom written consent is obtained will be assigned a five-digit screening code, consisting of the site number (e.g. 01) and a progressive number within the site: for example the first subject screened will be assigned the code 01-001, the second one 01-002 etc.
All screened subjects will receive the code irrespective of whether or not they will be randomized. If a subject discontinues from the study at any time, the code will not be re-used.
All subjects who will sign the informed consent and receive the screening code will be entered into a Subject's Register, containing the name and surname of the subjects and the date they have signed the consent form.
The randomization list will be generated by Latis S.r.l., using the PROC PLAN of SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). Once eligibility of a subject is established (see Inclusion/Exclusion Criteria) the study treatment will be assigned through

envelopes randomization system: the site will be provided with sealed envelopes, numbered in progressive number starting from R-001, containing the treatment kit to be assigned to the subject. The Investigator will open the first available envelope in progressive order. Inside the envelope there will be the kit number of the treatment to be assigned to that subject. Opened envelopes will be signed and dated by the Investigator and stored in the Investigator's File.
The Investigator will keep record of all enrolled subjects in the Subject's Enrolment Log: the subject screening number, the date of consent, the treatment assigned to the subject, if applicable, or the reason for not being randomized will be recorded.

6.2 BLINDING / EMERGENCY UNBLINDING

The study is designed as a double blind study and neither the subject nor the clinical site personnel (Investigator, sub-Investigator, study nurse, psychologists, pharmacist) will know which treatment is being administered. The identity of the treatments can't be revealed except in an emergency under the discretion of the Investigator.
The Principal Investigator will receive a study treatment identification key in the form of sealed envelopes containing the kit number and the corresponding treatment.
The envelope can be opened only in case of an emergency presenting the need to disclose the identification of the study treatment assigned to the subject, for the purpose of establishing the appropriate therapy. Once the code is broken for a subject, this subject shall be withdrawn from the study, with the completion of the final study evaluation, indicating the specific reason of the subject withdrawal.
The Study Monitor must be notified immediately by the Investigator of any emergency unblinding; the date and time, along with the reason for the unblinding, will be noted. Treatment codes will not be freely available to the Investigator or personnel monitoring the study until after the study completion and database lock.

6.3 STUDY DURATION AND END OF STUDY DEFINITION

Each participant will attend 4 visits over a total period of about 9 weeks: an initial screening phase of 7 to 1 days and then a treatment phase of 8 weeks.
Duration of the treatment: 8 weeks in a double blind scheme (food supplement versus placebo).
The enrolment period is foreseen to be of 2 months and the overall expected study duration is about 4-5 months.
Unless premature interruption occurs, the end of the study will be the closure visit at clinical site.

7. SELECTION AND WITHDRAWAL OF SUBJECTS

Subjects aged ≥60 years with no evidence of cognitive dysfunction will be enrolled at the Geriatric Division of the P.O. SS Filippo e Nicola, Avezzano, Italy.
No. 80 (including a possible 15% for screening failure and drop-out) subjects will be involved in this study.

7.1 INFORMED CONSENT PROCEDURE

Prior to the subject's enrolment into the study and before performing any study-related procedures, the Investigator - or its authorised delegate - shall obtain the subject's written, dated and signed informed consent to participate into the study and to the confidential disclosure, processing and transferring necessary documentation of the subject's health and personal data to the CRO, the Sponsor and its Affiliates, the competent Health Authorities and any other institutions (even if located outside the European Economic Area), as legally required and in accordance with the applicable privacy laws.
Institution and Investigator undertake to duly inform subjects about personal data processing and the relevant applicable privacy rights before their participation into the study.

Prior to be submitted to the subject, the Informed Consent form will be approved in the corresponding local language and in accordance with local laws and regulations by the IEC.
Subjects will be given information and fully comprehensive explanation in easily understandable terms of the study procedures, regarding the benefits, restrictions, discomforts, and risks in taking part in the study, the properties of the study product, the method of assignment to treatments, and any medically accepted and readily available treatment other than the study product.
Subjects will also be informed about the measures taken to ensure their confidentiality according to the pertinent legislation. The Investigator will provide the subject with an emergency telephone number.
After being duly informed and interviewed by the Investigator, the subject freely has to date and sign the ICF before being enrolled into the study and before undergoing any study procedure. The Investigator must store the original of the signed ICF in the Investigator's File, and the subject will be provided with a copy of it.
If a protocol amendment would affect the terms of the ICF, it will be revised to reflect the protocol change and submitted to IEC for approval.
The Investigator will ensure that this new consent form is signed by all subjects subsequently entered in the study and those currently in the study, if affected by the amendment.

7.2 INCLUSION CRITERIA

For inclusion in the study, all of the following inclusion criteria must be fulfilled:

1) Subjects aged ≥60 years.
2) Subjects who provide written Informed Consent to the study.

7.3 EXCLUSION CRITERIA

Subjects will not be considered eligible for this study if they fulfill any of the following exclusion criteria:

1) Subjects with cognitive dysfunctions or clinically significant coexisting medical conditions (cardiovascular disease, cerebrovascular events, overt dementia defined by MMSE <27 or other neurological disorders, thyroid disorders, or inflammatory diseases)
2) Subjects with a score on the Geriatric Depression Scale (GDS) >11 in order to avoid confounding due to the influence of concomitant depression on the performance on cognitive tests
3) Current smokers
4) Habitual users of antioxidant supplements (including vitamins C and E)
5) Habitual consumers of chocolate or other cocoa products (daily consumption of any amount)
6) Subjects under treatments with medications known to have antioxidant properties (including statins and glitazones) or to interfere with cognitive functions (including benzodiazepines and antidepressants)
7) Subjects with hypersensitivity to any component of the study medications.
8) Subjects who are participating in or having participated in another clinical trial within the previous three months.

7.4 WITHDRAWAL CRITERIA

The subject may withdraw from the study at any time without explanation, without losing the right to future medical care. The participation of the subject may, at any moment, be terminated by the Investigator, if considered appropriate.
Subjects who discontinue from the study early will complete an early termination visit (Visit 4).
Study treatment must be terminated during the study for any of the following reasons:

- Request of the subject (consent withdrawal);
- An AE occurs that, in the opinion of the Investigator, makes unsafe for the subject to continue in the study;
- Investigator deems it to be in the best interest of the subject to discontinue;

- Failure to comply adequately with the dosing, evaluations, or other requirements of the study

The Investigator must immediately notify the C.R.O (see C.R.O. officer contacts on page 37) by telephone or fax when a subject has been discontinued/withdrawn due to an AE.
The reason for the withdrawal must be well documented in the CRF.
Withdrawn subjects will not be replaced as already foreseen in the sample size estimation.
Any deviation from the protocol (to be classified as major or minor) will be accepted only in case of emergency and/or after a written agreement with the Sponsor.

8. TREATMENT OF SUBJECTS

Subjects will be randomly allocated to one of the following groups:

- Group I: mix of the four bioactive compounds (bacopa, lycopene, astaxanthin and vitamin B12) for 8 weeks;
- Group II: placebo for 8 weeks.

8.1 STUDY PRODUCTS

The product under investigation is a new food supplement composed by four bioactive ingredients: bacopa, lycopene, astaxanthin and vitamin B12. The product and its placebo will be administered to subjects in oral tablets to be taken once a day for 8 weeks.

Study product 1:
Name: food supplement (a mix of bacopa, lycopene, astaxanthin and vitamin B12)
Formulation: tablets
Route of administration: oral

Table 1: food supplement tablet composition

Average amounts of characterizing ingredients	Quantity (mg/dose)	% NRVs
Astaxanthin	160.000	15.610
Lycopene	100.000	9.756
Bacopa (Bacopa monnieri (L.) Pennel) aerial part e.s. tit. 50% bacosides	80.000	7.805
Cyanocobalamin (vitamin B12)	6.000	0.585
Microcrystalline Cellulose	619.434	60.433
Coating agent: hydroxypropylmethylcellulose	15.819	1.543
Antiagglomerating agents: crosslinked sodium carboxymethylcellulose	15.000	1.463

Antiagglomerating agents: polyethylene glycol	8.500	0.829
Antiagglomerating agents: magnesium vegetable stearate	8.000	0.780
Antiagglomerating agents: silicon dioxide	6.000	0.585
Dye: titanium dioxide	3.651	0.356
Antiagglomerating agent: vegetable stearic acid	2.434	0.237
Dye: oxide of iron (red)	0.150	0.015
Dry: Patent blue V	0.013	0.001
Total	**1025.000**	**100.000**

Study product 2:
Name: placebo
Formulation: tablets
Route of administration: oral

Table 2: placebo tablet composition

Average amounts of characterizing ingredients	Quantity (mg/dose)	% NRVs
Microcrystalline Cellulose	994.434	97.018
Coating agent: hydroxypropylmethylcellulose	15.819	1.543
Antiagglomerating agents: magnesium vegetable stearate	5.000	0.488
Dye: titanium dioxide	3.651	0.356
Antiagglomerating agents: silicon dioxide	3.000	0.293
Antiagglomerating agent: vegetable stearic acid	2.434	0.237
Antiagglomerating agents: polyethylene glycol	0.500	0.049
Dye: oxide of iron (red)	0.150	0.015
Dry: Patent blue V	0.013	0.001
Total	**1025.000**	**100.000**

Tablets of the food supplement and placebo will be manufactured and packaged (primary and secondary packaging) by Fine Foods & Pharmaceuticals N.T.M. S.p.A. Zingonia-Verdellino (BG), Italy. Study products will be then labeled, supplied to the clinical site, returned from the clinical site and destroyed by Euromed Clinical Supply Services (EUROMED), Cantù (CO) - Italy
No difference will be detectable in the presentation of the food supplement and placebo tablets.

Tablets will be packaged in blisters (primary packaging) and in boxes (secondary packaging).
Each box will contain 3 blisters with 15 tablets each. The subject's kit will be composed by two boxes, named 1 and 2, in order to cover the 4 + 4 weeks study treatment periods. Subjects will receive at each visit enough tablets for daily treatment (one tablet/day) until the next visit. The number of tablets provided will be in large excess (45 tablets), with respect to the hypothetical need (28-31 tablets for 4 weeks ± 3 days at each phase).
Blisters and boxes will be labelled according to Annex 13 requirements. Boxes will have a tear-off portion of label to be attached on the appropriate section of the CRF.

Each treatment will be defined by a three-digit number and by the numbers 1 and 2 to indicate the first and the second box to be used (i.e. treatment 015-1 and 015-2, 034-1 and 034-2). Treatment numbers will be randomized.

8.2 HANDLING AND STORAGE

The study product shall be carefully stored at the study site, in a safe area and separately from other drugs/products. It shall not be exposed to direct sunlight or heat.
The Investigator's staff shall maintain a record of the study product delivery to the study site and inventory at the study location.
After study conclusion, all unused study product shall be returned and destroyed by EUROMED after written Sponsor approval.

8.3 STUDY PRODUCT ACCOUNTABILITY AND RETURN

The Investigator is responsible of ensuring accountability of the study product, including reconciliation of the study products and maintenance of records.
Upon receipt of the study products, the Investigator (or designee) will check the contents and acknowledge receipt by signing (or initialing) and dating the documentation provided by the C.R.O and returning it to the C.R.O. A copy will be retained in the Investigator File.
The dispensing of the study product will be carefully recorded on the appropriate accountability forms provided by the C.R.O and an accurate accounting will be available for verification by the Study Monitor at each monitoring visit.
Study product accountability records will include:

- Confirmation of study product receipt at the clinical site.
- The inventory at the site of study product provided by the C.R.O.
- The intake of doses by each subject.
- The return to EUROMED or alternative disposition of unused study product.

The Investigator should maintain records that adequately document:

- That the subjects were provided the doses specified by the clinical study protocol/amendment(s), and

- That all study products provided by EUROMED was fully reconciled.

To the purpose of accountability, the subject will be instructed to always return the unused containers at each visit.

Unused study product must not be discarded or used for any purpose other than the present study. Study product that has been dispensed to a subject must not be re-dispensed to a different subject.

The Study Monitor will periodically collect the study product accountability forms and will check all returns (both unused and used containers) before arranging for their return to EUROMED.

8.4 TREATMENT COMPLIANCE

The subject compliance for study treatment period is calculated by the following formula: % compliance = number of tablets actually taken x 100 / expected number of tablets which should have been taken.

The global subject compliance will be calculated as the percentage of the number of tablets actually taken by the subject over the number of tablets expected to be taken.

The number of tablets actually taken will be calculated as the difference between the number of tablets handled out to the subject and the number of unused tablets returned or declared lost by the subject.

The expected number of tablets taken will be calculated using the difference (in days) between Visit 3 and Visit 2 and between Visit 4 and Visit 3. A subject that has taken at least 80% and no more than 120% of the required product intake since the last visit will be considered compliant.

8.5 ADMINISTRATION OF STUDY TREATMENT

Subjects will be randomly allocated to one of the following groups:

- Group I: mix of the four bioactive compounds (bacopa, lycopene, astaxanthin and vitamin B12), 1025,00 mg/daily);
- Group II: placebo, 1025,00 mg/daily.

Both treatments, the food supplement and placebo, shall be taken once a day for 8 consecutive weeks: one tablet a day, with a glass of water, possibly at the same time of the day and away from meals.

8.6 CONCOMITANT TREATMENTS

Any medications (other than those excluded by the clinical study protocol) that are considered necessary for the subjects' welfare and will not interfere with the study product may be given at the Investigator's discretion.

The Investigator will record all concomitant medications taken by the subject at study inclusion and during the study, in the appropriate section of the CRF.

Any additional concomitant therapy that becomes necessary during the study and any change to concomitant treatments must be recorded in the corresponding section of the CRF, noting the name, dose, duration and indication of each product.

8.7 PROHIBITED PRIOR AND CONCOMITANT TREATMENTS

At screening and during the study the following concomitant treatments are not allowed:

- Antioxidant supplements (including vitamins C and E)
- Medications known to have antioxidant properties (including statins and glitazones)
- Medications known to interfere with cognitive functions (including benzodiazepines and antidepressants).

Habitual use of chocolate or other cocoa product should be avoided during the study.
If the treatment with one or more of the above mentioned products becomes necessary during the study, then the subject will be withdrawn from the study.

9. ASSESSMENT OF EFFICACY

9.1 EFFICACY PARAMETERS

Cognitive functions, mood state, self-perceived well being and sexual satisfaction will be assessed at baseline and after 8 weeks of regular consumption of the mix of the four antioxidant compounds or placebo.

Blood pressure, metabolic variables and plasma markers of oxidative stress will be assessed at baseline, after 4 and after 8 weeks of regular consumption of the antioxidant compounds or placebo.

9.1.1 Cognitive Performances Assessment

Cognitive performances will be evaluated by TMT, VFT, MoCA, MMSE and AVLT.

-Trail Making Test (TMT) is a frequently used neuropsychological test because of its sensitivity to brain damage. It explores visual-conceptual and visual-motor tracking. TMT is administered in two parts. Part A is a visual-scanning, timed task where participants are asked to connect with lines 25 circles numbered from 1 to 25 as quickly as possible. The test is terminated after 5 minutes even if not completed. In Part B participants are asked to connect circles containing numbers (from 1 to 13) or letters (from A to L) in an alternate numeric/alphabetical order. The test is terminated in every case after 10 minutes even if not completed. The TMT B-A score calculated as the difference between TMT-B and TMT-A times is considered a measure of cognitive flexibility relatively independent of manual dexterity.

-Verbal Fluency Test (VFT) is a short test of verbal functioning. Participants are given 1 min to produce as many unique words as possible within a semantic category (category fluency) or starting with a given letter (letter fluency). The participant's score in each task is the number of unique correct words.

-Montreal Cognitive Assessment (MoCA) evaluates a broader array of cognitive domains (e.g., attention/executive functioning, visuospatial abilities and language) and it has been demonstrated to be able to detect cognitive impairment with scores ranging from 0 to 30.

-Mini Mental State Examination (MMSE) is a widely used screening tool for cognitive impairment and covers five areas of cognitive function including orientation, attention, calculus, recall and language with scores ranging from 0 to 30.

-Rey Auditory Verbal Learning Test (AVLT) is a neuropsychological assessment designed to evaluate the nature and severity of memory dysfunction and to track changes in memory function. The examiner reads aloud a list of 15 words at the rate of one per second. The participant is then asked to repeat all words from the list that she/he can remember. This procedure is carried out a total of five times. After a 15-minute delay, the participant is again asked to recall as many words as possible from the first list. The participant is then requested to read a list of words and asked to indicate whether each word was from the first list. The score for each trial is the number of words correctly recollected.

9.1.2 Mood States Assessment

Mood states will be assessed by POMS.

-Profile of Mood Stated (POMS) is a widely used tool in assessing mood states that has already been validated for Italy. Higher scores reflect mood decrements, except for the vigor subscale, where higher scores reflect improved mood. Taking into consideration the two components characteristic of the POMS that measure negative affect and positive affect, respectively, the data collected with the questionnaire will be analyzed both as global score and by separately considering a Positive Affect Scale (PAS-POMS, 48 items, score ranging from 0 to 192) and a Negative Affect Scale (NAS-POMS, 10 items, score ranging from 0 to 40).

9.1.3 Psychological Well-Being Assessment

Psychological well-being will be assessed by GHQ-12.

-General Health Questionnaire (GHQ-12) is a relevant instrument for measuring psychological well-being. It has been extensively evaluated in terms of validity and reliability as a one dimensional indicator of the severity of psychological morbidity, and it has already been validated for Italy.

9.1.4 Sexual Satisfaction Assessment

Sexual satisfaction will be evaluated by the NSSS.

-New Sexual Satisfaction Scale (NSSS) is a 20 item, multidimensional, composite measure of sexual satisfaction based on a five-dimension, conceptual model that emphasized the importance of multiple domains of sexual behavior including sexual sensations, sexual awareness and focus, sexual exchange, emotional closeness, and sexual activity.

9.1.5 Blood Pressure, Metabolic Variables and Plasma Markers Of Oxidative Stress

-Clinic systolic and diastolic blood pressure levels will be recorded in the morning, using a validated oscillometric device with appropriately sized cuffs on the non-dominant upper arm after 5 min resting in a seated position; the first blood pressure measurement will be discarded and the subsequent three consecutive blood pressure readings, taken at 3-min intervals, will be recorded. The average of these latter measures will be considered for statistical analysis. [O'Brien et al., 2005].

-Metabolic profile will be evaluated by the following variables:

1. glucose (normal range 60-100 mg/dL)
2. insulin (normal range 2.7-10.4 mcUI/mL)
3. HOMA-IR (derived from glucose and insulin)
4. total cholesterol (normal range 150-200 mg/dL)
5. LDL cholesterol (normal range 40-130 mg/dL)
6. HDL cholesterol (normal range 35-90 mg/dL)
7. triglycerides (normal range 60-170 mg/dL)
8. uric acid (normal range 3.5 – 7.0 mg/dL)

-***Oxidative stress*** will be evaluated by measuring circulating levels of 2 soluble biomarkers:

A. 8-iso-Prostaglandin F2alpha (no predefined normal range)
B. Plasma malondialdehyde (no predefined normal range)

9.2 STUDY PROCEDURES

The study will last approximately 9 weeks for each subject.
A total of 4 study visits will be performed as follows.

VISIT 1 (screening, day -7 to Visit 2):

Subjects who provide written informed consent will undergo screening assessments to evaluate eligibility for the study. Subjects who meet all inclusion criteria and none of the exclusion criteria will be eligible for the study and will undergo to the following evaluations:

1) Collection of medical history and information about eventual concomitant medications
2) Complete physical evaluation including
    a) anthropometric parameters (height, weight, waist circumference)
    b) cardiovascular system
    c) respiratory system
    d) gastrointestinal system
    e) respiratory system
3) Blood pressure measurement
4) MMSE
5) GDS.

Subjects eligible for the study will meet with a dietician in order in order to evaluate current diet habits and correct any nutritional insufficiencies. Participants will be then instructed to maintain their usual lifestyle and intake of fruits and vegetables and to avoid any food supplement.

VISIT 2 (randomization, 7 days after V1):

Subjects eligible for the study will undergo to the following evaluations:

1) Collection of information about eventual concomitant medications
2) Complete physical evaluation including
    a) anthropometric parameters (weight, waist circumference)
    b) cardiovascular system
    c) respiratory system
    d) gastrointestinal system
    e) respiratory system
3) Blood pressure measurement
4) Neuropsychological evaluation (TMT, VFT, MoCA, MMSE, AVLT)
5) Evaluation of mood state (POMS)
6) Evaluation of quality of life (GHQ-12)
7) Evaluation of sexual satisfaction (NSSS)
8) Blood samples collection to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid)

9) Blood samples collection to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde).
10) Adverse events check and evaluation

Subjects will be randomly assigned to two parallel groups in a 1:1 ratio and treated with a mix of the four bioactive compounds (bacopa, lycopene, astaxanthin and vitamin B12, – (1025,00 mg/daily) or placebo (1025,00 mg/daily). Subjects will intake one tablet/day for about 8 weeks.

During Visit 2 one box of study treatment (containing 45 tablets) will be given to each subject.

VISIT 3 (treatment period, 30 days ± 3 days after V2):
Subjects will undergo to the following evaluations:
1) Collection of information about eventual concomitant medications
2) Complete physical evaluation including
    a) anthropometric parameters (weight, waist circumference),
    b) cardiovascular system
    c) respiratory system
    d) gastrointestinal system
    e) respiratory system
3) Blood pressure measurement
4) Blood samples collection to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid)
5) Blood samples collection to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde)
6) Adverse events check and evaluation
7) Study product return/compliance check of first month of therapy.

During Visit 3 another one month of treatment of study product (45 tablets) will be given to each subject.

VISIT 4 (final visit, 30 days ± 3 days after V3):
Subjects will undergo to the following evaluations:
1) Collection of information about eventual concomitant medications
2) Complete physical evaluation including
    a) anthropometric parameters (weight, waist circumference)
    b) cardiovascular system
    c) respiratory system
    d) gastrointestinal system

e) respiratory system

3) Blood pressure measurement
4) Neuropsychological evaluation (TMT, VFT, MoCA, MMSE, AVLT)
5) Evaluation of mood state (POMS)
6) Evaluation of quality of life (GHQ-12)
7) Evaluation of sexual satisfaction (NSSS)
8) Blood samples collection to assess metabolic parameters (glucose, insulin, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, uric acid)
9) Blood samples collection to assess plasma markers of oxidative stress (8-iso-Prostaglandin F2alpha, malondialdehyde)
8) Adverse events check and evaluation
7) Study product return/compliance check of second month of therapy.

10. ASSESSMENT OF SAFETY

All adverse events will be duly assessed for identifying any emergent safety finding.

10.1. SAFETY DATA MANAGEMENT

10.1.1 Adverse Event (AE)

Any untoward medical occurrence in a subject or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment.
An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

10.1.2 Product Relationship

The relationship between an AE and study products will be judged according to the following categories:

1. **Certain**: The AE occurs in a plausible time relation to the administration of the product and cannot be explained by a concurrent disease or other drugs or chemicals. The response to withdrawal of the product (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.
2. **Probable**: The AE occurs in a reasonable time relation to the administration of the product, it is unlikely to be attributed to a concurrent disease or other drugs or chemicals and it follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information (AE reappearance after product reintroduction) is not required to fulfil this definition.

3. **Possible**: The AE occurs with a reasonable time relation to the administration of the product, but it could also be explained by a concurrent disease or other drugs or chemicals. Information on product withdrawal (dechallenge) may be lacking or unclear.
4. **Unassessable**: The relationship cannot be judged, because of the information is insufficient or contradictory and cannot be supplemented or verified.
5. **Unlikely**: A causal relationship cannot be definitively ruled out, but
   – other drugs, chemicals, or underlying disease provide plausible explanations and/or
   – the temporal relation to the administration of the product makes a causal relation improbable.
6. **Not Related**: Any of the following are present:
   – existence of a clear alternative explanation, and/or
   – unreasonable temporal relationship between product and event, and/or
   – non-plausibility.

10.1.3 Adverse Reaction (AR)

An AR is any untoward and unintended response to an investigational product related to any dose administered.

The definition implies a reasonable possibility of a causal relationship between the event and the investigational product. This means that there are facts (evidence) or arguments to suggest a causal relationship.

An AR is considered any AE for which the relationship is considered as:

1. Certain
2. Probable
3. Possible
4. Unassessable

An AE is **not** considered as AR when the relationship is judged as:

5. Unlikely
6. Not related

10.1.4 Seriousness

An AE/AR is considered **Serious** when:

- results in death;
- is life-threatening;

Note: Life-threatening is considered any AE in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

- requires insubject hospitalisation or prolongation of existing hospitalisation;

- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect;
- is another medically important condition that may jeopardise the subject or may require intervention to prevent one of the outcomes listed above. Any suspected transmission of an infectious agent via a medicinal product is considered serious and should be assessed under the category of medically important events in the absence of other seriousness criteria.

An AE/AR is considered **Non-serious** when it does not fulfill the conditions for the definition of Serious AE/AR.

10.1.5 Adverse Event/Adverse Reaction Intensity

The intensity level of a Serious or a Non-serious AE or AR is attributed according to the following definitions:

- **Mild**: does not interfere with routine activities; in case of laboratory tests, when there is a mild abnormality.
- **Moderate**: interferes with the routine activities; in case of laboratory tests, when there is a moderate abnormality.
- **Severe**: makes it impossible to perform routine activities; in case of laboratory tests, when there is a significant abnormality.

10.1.6 Adverse Event/Adverse Reaction Expectedness

An AE/AR is considered **Unexpected** when the nature, severity, or outcome of the AE/AR is not consistent with the information known about the product.

10.1.7 Collection, Recording and Reporting of AEs

At each visit the Investigator will **collect and assess** any occurred subjective or objective AE occurred to each subject after his/her signature of the informed consent.

The Investigator should manage as AE any laboratory test abnormality (newly occurring after the study product administration or worsening of previously known abnormalities) considered as clinically relevant: i.e. values significantly above or under normal range or which require an intervention or diagnostic tests, or may result in the study product discontinuation.

Any AE communicated by the subject or by the subject's relatives or delegates through phone calls, letters or emails will also be collected and assessed.

The Investigator shall record on the respective CRF AE recording pages/AE form any recognised AE, both serious and non-serious, whether or not thought to be product-related, observed in or reported by the subject (or relatives/delegates), specifying the judgement on the causal relationship with the study treatment.

Any available information and diagnostic measure (laboratory and instrumental tests, procedures, etc.) shall be recorded in and/or attached to the concerned CRF pages/sections.
The Investigator must report all the collected information on any Serious AE (whether or not thought to be related to the investigational product), providing the concerned SAE reporting form by email or fax, after the first knowledge of the occurrence of the case, to:

CRO Officer: Laura Michellini
Email: michellini@latiscro.it
Fax: + 39 010 540699
Tel: +39 010 562234
Mobile: +39 347 4785898

When relevant, also the CRF pages concerning medical history, concomitant medication, and laboratory tests will be placed at Sponsor disposal by email or fax.

10.1.7.1 Management of Serious AEs (SAEs) including laboratory abnormalities

- ***Reporting Duties of the Investigator***

The Investigator must report all the collected information on any SAE (whether or not thought to be related to the investigational product), as above specified, **no later than 24 hours** after the first knowledge of the occurrence of the case.
Any further information and supporting documentation that become available (copies of laboratory reports, tests, procedures, autopsy evidence of the cause of death, etc.) shall be provided no later than 24 hours after the knowledge, by the Investigator to the CRO by email or fax, to be forwarded to the Sponsor.
The Investigator must also comply with the local applicable obligation(s) on the reporting of ARs to the local concerned Regulatory Authority/Ethics Committee.

- ***Reporting Duties of the Sponsor***

The Sponsor shall ensure that all relevant information about any SAE (whether or not thought to be related to the investigational product), is expeditiously reported to the competent Authority and Ethics Committee as required, with these deadlines after the first knowledge, intended as the day when the CRO receives the notification of the SAE:

- Fatal and life threatening unexpected cases, no later than 7 days;
- Other unexpected serious cases, no later than 15 days.

The Sponsor shall ensure that all relevant information and supporting documentation that subsequently becomes available, is also expeditiously reported as follow-up information according to the above mentioned deadlines.
Since the study is ***blinded,*** the subject's code will be broken before the expedited reporting to the Competent Authority and the Ethics Committee.

Furthermore the following safety issues will be subjected to expedited management for the identification of possible necessary actions:

- SAEs associated with the study procedures
- Potential clinically significant findings emerging from non-clinical studies
- An anticipated end or suspension for safety reasons of another study with the same study product.

When appropriate and applicable the Sponsor will arrange the adequate information also to the Investigators.

10.1.7.2 Management of Non-serious AEs (NSAEs) including laboratory abnormalities

- **Reporting Duties of the Investigator**

The Investigator must record all the collected information on any NSAE (whether or not thought to be related to the investigational product) on the appropriate section of the CRF.

10.1.8 MANAGEMENT OF ANY LABORATORY ABNORMALITY

Any laboratory test abnormality which is considered by the Investigator as AE is to be managed as above detailed (see 10.1.7).

10. STATISTICS

10.1 STATISTICAL METHODS (BLINDING AND RANDOMISATION)

Once eligibility will be established, subjects will be randomized to receive either a mix of the four bioactive compounds or placebo in a 1:1 ratio and a double-blind manner.

Descriptive statistics of all relevant variables will be performed. Continuous variables will be summarized by the number of subjects (N), mean, standard deviation, median, minimum, maximum. Where appropriate, 95% confidence intervals for the target variables will be estimated. Categorical variables will be summarized by the number (N) and the proportion of subjects (%).

The significance level of statistical tests will be set at 0.05. Details of statistical analysis are provided in the following paragraphs. Further details will be given in a Statistical Analysis Plan that will be issued before database lock.

No adjustment for multiplicity is needed. Missing values will not be replaced. No interim analysis is planned.

The statistical analysis will be performed using SAS 9.4 for Windows (SAS Institute Inc., Cary, NC, USA).

10.2 DETERMINATION OF SAMPLE SIZE

The primary objective of this study will be assessed using three primary variables (changes in TMT-B, TMT-A and TMT B-A) in a hierarchical order. According to the "Points to consider on multiplicity issues in clinical trials", issued by the Committee for Proprietary Medicinal Products (CPMP - 2002), no sample size adjustment for multiplicity is needed. The study will be based on an estimated sample size of 68 subjects, with a ratio of 1:1 for the 2 treatment groups, which has been calculated to be adequate to achieve 90% power to detect a large effect size (f=0.40) using an ANCOVA model with a baseline covariate and an α of 0.05 between treatment and control (G*power version 3.1.9.2). To consider a possible 15% dropout rate, 80 subjects will be enrolled.

10.3 ANALYSIS POPULATIONS

The study will be analyzed using an Intent-to-Treat (ITT) approach. All randomized subjects receiving at least a treatment dose and having the post-randomization efficacy evaluation will be included in the ITT population for efficacy analysis.

All subjects without protocol deviations affecting the primary endpoint will be included in the Per-Protocol (PP) analysis. Protocol deviations affecting the protocol can include deviations to the treatment dose or assessment schedule, violations of some inclusion/exclusion criteria, etc. Detailed list of these deviations will be created and included in the SAP.

All randomized subjects receiving at least a treatment dose will be included in the safety analysis.

10.4 ANALYSIS VARIABLES

10.4.1 Primary Efficacy Endpoint

The primary efficacy endpoints are the changes of TMT scores from baseline (V2) to 8 weeks of treatment (V4), analyzed in the following hierarchical order: TMT-B, TMT-A and TMT B-A.

10.4.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints of the study are:

a) changes of VFT score from baseline (V2) to 8 weeks of treatment (V4);
b) changes of MoCA score from baseline (V2) to 8 weeks of treatment (V4);
c) changes of MMSE score from baseline (V2) to 8 weeks of treatment (V4);
d) changes of AVLT score baseline from baseline (V2) to 8 weeks of treatment (V4);
e) changes of GHQ-12 score baseline from baseline (V2) to 8 weeks of treatment (V4);
f) changes of POMS score from baseline (V2) to 8 weeks of treatment (V4);
g) changes of NSSS score from baseline (V2) to 8 weeks of treatment (V4);

h) changes of metabolic parameters (glucose, insulin, HOMA-IR, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and uric acid from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4);
i) changes of circulating levels of 8-iso-Prostaglandin F2alpha and malondialdehyde from baseline (V2) to 4 weeks of treatment (V3) and from baseline (V2) to 8 weeks of treatment (V4).

10.4.3 Exploratory Endpoints

Exploratory endpoints of this study are:

a) correlation between changes of cognitive scores (TMT, VFT, MoCA, MMSE, AVLT) and changes of blood pressure and metabolic parameters including indices of oxidative stress;
b) quantify the effect of time in the change for the primary endpoint.

10.5 STATISTICAL ANALYSIS

Efficacy analysis

The primary variables (changes of TMT-B, TMT-A and TMTB-A scores) will be analyzed using an ANCOVA model with the treatment group (main effect) and baseline as covariates. A fixed sequence multiple test will be performed, with the following hierarchical order: 1) changes of TMT B, 2) changes of TMT A and 3) changes of TMT B-A scores.

All the other continuous variables (changes of test scores, changes of metabolic parameters, changes of circulating levels of 8-iso-Prostaglandin F2alpha and malondialdehyde) will be analyzed using an ANCOVA model with the treatment group (main effect) and baseline as covariates. As additional covariate for testing the homogeneity of the regression coefficients the interaction term of treatment*baseline will be included in the model and removed if not significant.

Categorical variables (gender, eventually present cardiovascular risk factors or pharmacological treatment) will be tested by using a Chi-Square test or the Fisher exact test if necessary.

Explorative analysis

- Pearson correlation will be used to evaluate correlations between changes of cognitive scores (TMT scores, VFT, MoCA, MMSE, AVLT) and changes of blood pressure and metabolic parameters including indices of oxidative stress. Spearman nonparametric correlation will be also applied when one or both of the variables are not assumed to be normally distributed and interval (but are assumed to be ordinal).
- in order to evaluate and quantify the effect of time itself (i.e. 8 weeks after the treatment intake) in the change for the primary endpoint a two-way analysis of variance (treatment*time) with time as a repeated measure will be also implemented.

Safety analysis

All adverse events will be duly assessed for identifying any emergent safety finding.

Adverse events will be coded using the last updated version of the Medical Dictionary for Regulatory Activities (MedDRA) dictionary to give a preferred term (PT) and a system/organ class term (SOC) for each event.

The number of AEs, study product-related AE, serious AE, severe AE will be summarized by treatment arm. The number of subjects who experienced at least one AE and the number of subjects withdrawn due to AE will also be summarized.

AEs occurred after the first treatment intake will be considered Treatment Emergent Adverse Events (TEAEs), selected and analyzed separately.

For each SOC and preferred term, summaries will be made with respect to the proportion of subjects having at least one occurrence of that event during the trial and the total number of events. The incidence of AEs in each treatment arm will be presented overall, by SOC and preferred term, and additional grouping by severity and relationship to the trial treatment. The comparisons will be analyzed using chi-square test.

10.6 SUBGROUP ANALYSIS

No subgroup analysis is planned.

10.7 PROTOCOL VIOLATIONS AND BLIND REVIEW

Protocol deviations will be registered during the course of the study. Protocol deviations can be identified in various ways: during onsite monitoring, during remote data checking, during or after data entry, thanks to data checks on CRFs or automated data checks during or after data entry. The impact of each deviation and the opportunity to drop subjects following a deviation will be discussed.

Blind data review will be performed prior to database lock, to assess data completeness and protocol deviations.

10.8 STATISTICAL ANALYSIS PLAN

The reference document for the Statistical Analysis Plan is the ICH Topic E9 Statistical Principles for Clinical Studies: Note for Guidance on Statistical Principles in Clinical Studies.

Further details of the statistical analyses will be provided in the Statistical Analysis Plan (SAP), which will be finalized prior to breaking the blind.

11. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The Investigator agrees to allow Sponsor/CRO auditors/monitors to have direct access to his/her study records for review, being understood that the auditors/monitors are bound by professional secrecy, and as such will not disclose any personal identity or personal medical information.

The Sponsor and the CRO must ensure that the Investigator keeps secret from third parties any confidential information disclosed or provided by the Sponsor and regarding the Sponsor and its study-related products. The Investigator agrees to use such information only to accomplish the present study tasks and not to use it for any other purposes without the prior written consent by the Sponsor. A confidentiality agreement will be signed between the Sponsor and the main Investigator.

Study documents provided by the Sponsor will be kept appropriately to ensure their confidentiality.

12. QUALITY CONTROL AND QUALITY ASSURANCE

12.1 DATA QUALITY CONTROL/STUDY MONITORING

The study will be monitored by CRO's adequately qualified and trained clinical Monitor. Before the start of the study, the Clinical Monitor responsible for the study site has the task to assess the adequacy of the study site and the staff involved. After start, the study will be monitored on a regular basis throughout the study period to ensure the proper conduct of the clinical study.

The purposes of clinical study monitoring are to verify that the rights and well-being of the study subjects are protected, that the reported study data are accurate, complete and verifiable against the source documents, and that the study is conducted in accordance with the current protocol, Good Clinical Practice guideline and applicable regulatory requirements.

During the monitoring visits, Monitors will verify the following including but not limited to: subject's informed consent, subject's eligibility, safety data and reporting, subject's questionnaires, quality of source documents and CRF data against subject's medical records.

If inconsistencies are identified, the corresponding corrections to the CRF data will have to be made by the Investigator or designated person. Monitors will also check treatment compliance, accrual, study product handling, including dispensing procedures and accountability logs, delegation of responsibilities within the Investigator's team, relevant communications with family doctors, if any, ancillary equipment and facilities, including refrigerators and freezers, local labs, etc.

The Investigator and other site staff involved in the study must allocate enough time to the Monitor at these visits.

12.2 CASE REPORT FORMS

Data collected during the study will be recorded in the Case Report Form (CRF), which is confidential. Data reported on the CRF have to be consistent with the source documents. The Investigator must ensure the accuracy, the completeness and the consistency of the data entered in the CRF.

On the CRF, subjects will be identified by the subject number, assigned at the Screening Visit. The subject number will be a number composed by the site code (01) and a progressive number (001, 002, 003…etc).

During each subject's study visit, the Investigator or designee will collect and report study data in the relevant subject's chart, documenting all significant observations.

Any contact with the subject via telephone or other means that provides significant clinical information must be documented in the source data and will be promptly entered in the CRF.

A paper CRF will be used for recording subject's study data.

The CRFs must be completed for all subjects who have been included in the study. The Investigator will review all CRFs and sign and date them for each subject, verifying that the information is complete, true and correct.

All CRF entries are to be done in black ballpoint ink. Changes and/or additions to data entered on original CRFs must be made in the following manner: the original entry will be lined out with a single line through the error (neither erasures nor correction fluid may be used) so as to leave it legible. The correction will be entered, initialed, and dated by the person making the correction. The Investigator or study personnel authorized by the Investigator may enter corrections on the original CRFs.

All fields on the CRF must be completed as applicable.

Subjects will be provided with paper questionnaires. Such documents will be filled by the subject during the study, to record data concerning cognitive performance, mood states, psychological well-being and sexual satisfaction.

It is responsibility of the Investigators to instruct the study participants on how to fill in questionnaires in a clear way and preferably in black ball-point pen. The questionnaires are anonymous, each subject is identified through the subject screening number. At the end of the study a copy of all questionnaires will be stored in the Investigator's File. It is responsibility of the Investigators to correctly enter the data collected on the questionnaires in the relevant sections on the e-CRF. Questionnaires will be considered source data.

After the CRFs are approved and signed by the Investigator, the monitor will collect and forward them to the Data Management Department where they will be processed. Data Clarification Forms generated by Data Management after data entry will be sent to the study site for resolution. The Investigator is responsible for the review and approval of all query resolutions.
The data collected by CRFs will be entered in a dedicated database under the responsibility of Latis. The access to the database will be controlled by user-specific account and password combinations and audit trailed. Checks to assist during the data entry and to assess the appropriateness and consistence of data will be developed.

12.3 QUALITY ASSURANCE

Upon request by the Sponsor, on-site study audits may be conducted in order to ensure the study is in compliance with GCP, applicable regulatory requirements, and the study protocol. The auditing activities may also be conducted after study completion.
In addition, Regulatory Authorities may wish to conduct on-site inspections (during the study or after its completion). If a Regulatory Authority notifies the Investigator of an inspection or visits the site unannounced for purposes of conducting an inspection, the Investigator must inform the Sponsor and CRO immediately. The Investigator will make all efforts to facilitate the conduct of the audits and inspections giving access to all necessary facilities, data and documents.
Any result or information arising from the inspection will be immediately communicated by the Investigator to the Sponsor. The Investigator will take all appropriate measures required by the Sponsor to implement corrective actions for all problems found during audits or inspections.

13. ETHICS ASPECTS

This study will be carried out in compliance with the study protocol, the recommendations on biomedical research on human subjects of the Declaration of Helsinki, International Conference of Harmonisation – Good Clinical Practice (ICH-GCP) Guidelines, EU-Directive 2001/20 and national requirements of the participating country.
All clinical work conducted under this protocol is subject to GCP rules. This includes audits/inspections by the Sponsor, and/or by national/international Health Authority representatives at any time. All Investigators must agree to the inspection of the study site, facilities, and of study related records by the Health Authority representatives and/or by the Sponsor, and/or its delegates, which must be performed in accordance with national laws concerning personal data protection.

13.1 ETHICS COMMITTEE

Before starting the study in a study site, study protocol and relevant documentation

(Subject information leaflet, the informed consent Form and the study protocol) must be submitted to and approved by the Independent Ethics Committee (IEC) of the participating centre. The study will be then notified to the Competent Authority (CA).
In addition, all local national legal requirements for the conduct of a clinical study have to be followed prior to the start of the study. Any amendment to the protocol, before implementation, will be submitted to the IEC for approval, after prior discussion between the Sponsor and the Co-ordinating Investigator.
The CA and IEC will be informed about any changes in the study protocol, the end of the study, or the premature study termination as appropriate and within the requested time period.

13.2 SUBJECT'S INSURANCE

For subjects participating in the study, Sponsor has stipulated an insurance policy in accordance with local regulatory requirements.
Details on the insurance company, the insurance number and conditions will be made available to subjects in the ICF and/or provided as a separate document, in accordance with national requirements.
A copy of the insurance certificate will be provided to each Investigator and will be filed in the Investigator's File at the sites and in the study's Trial Master File (TMF).
The Investigator must notify to Sponsor immediately upon notice of any claims or lawsuits.

14. DATA PROTECTION LAWS COMPLIANCE

By signing the study protocol, the Institution and the Investigator (including their appointed staff) acknowledge that the performance of the study will imply processing of personal data. Personal data processing is regulated by the Sponsor national legislation as well as local laws (Italy). The Sponsor specifies that strict compliance with the applicable data protection laws by any parties and relevant employees who take part in the study will be an essential condition for the appointment of and the collaboration with research institutions, investigators and the CRO.
The parties shall acknowledge that according to the applicable privacy laws, Sponsor and Institution/s will act as independent data controllers while CRO and Investigator/s will act as data processors.
Given the sensitive nature of data processed in the frame of the Study, the parties undertake to take adequate safety measures (physical, logical, organizational, technical, etc) to warrant that data will always be processed safely and in compliance with the Italian D.Lgs.196/03.
Investigator and Institution (including their personnel) shall comply with the applicable privacy laws on the protection of personal data.
Investigator must assure the subject's anonymity will be maintained. The Investigator will keep a separate log of the subject's study numbers, names, addresses and telephone

numbers. The Investigator will maintain this for the longest period of time allowed from own institution and in any case, till further communication from Sponsor.

15. DATA HANDLING AND RECORDS KEEPING

The Investigator should keep all study-related documents, as specified in ICH/GCP Section 8 “Essential Documents for the Conduct of a Clinical Trial” and all study documents as specified by the applicable regulatory requirement(s), in the Investigator's Trial Center File.

The Investigator will take measures to prevent accidental or premature destruction of these documents.

Essential documents must be retained for minimum 7 years.

If the responsible Investigator retires, relocates, or for other reasons withdraws from the responsibility of keeping the study records, custody must be transferred to a person who will accept the responsibility.

The Sponsor must be notified in writing of the name and address of the new custodian.

If it becomes necessary for the Sponsor and/or a Regulatory Authority to review any documentation related to this study, the Investigator must permit access to such documentation.

Any difficulty in storing original documents should be discussed with the Sponsor personnel prior to initiation of the study.

The protocol must be read thoroughly by everybody whom the information therein concerns and the instructions must be exactly followed.

Changes in the study protocol will require a protocol amendment. Such amendments will be agreed upon and approved in writing by all signatories of the protocol. If amendments are substantial, i.e. are likely to have an impact on the safety of the subjects, or to change the interpretation of the scientific documents in support of the conduct of the study, or if they are otherwise significant, the IEC has to approve these amendments before implementation.

Changes which have no significant impact on medical or scientific validity of the study will be agreed upon and approved in writing by all signatories of the protocol and the IEC will be notified of this protocol amendment.

Any substantial amendments of the protocol will be integrated in an updated study protocol. The Principal Investigator will ensure full compliance with the updated study protocol.

16. PUBLICATION POLICY AND RESULTS

By signing the study protocol, the Investigator (and his/her appointed staff) affirms that any information and all the study documents provided by the Sponsor will be maintained strictly confidential.

None of this material may be disclosed to any party not directly involved in the study without written permission from Sponsor.

The Investigator will supply the Sponsor with all the data/results from the study.

All information concerning the study, the study product as well as data and results of the study are confidential and property of the Sponsor. The Sponsor will prepare the final report, including the statistical and clinical evaluations. The Investigator's agreement and signature will be obtained and a copy will be provided to the Investigator.

Sponsor reserves the exclusive right to publish and present data and results of the present study at scientific meetings, or to submit these clinical study data to national and international Regulatory Authorities. The Investigator may not use the results of this study for publication or presentation without written authorization from Sponsor.

17. REFERENCES

Agca CA, Tuzcu M, Gencoglu H, Akdemir F, Ali S, Sahin K, Kucuk O. Lycopene counteracts the hepatic response to 7,12-dimethylbenz[a]anthracene by altering the expression of Bax, Bcl-2, caspases, and oxidative stress biomarkers. Pharm Biol. 2012 Dec;50(12):1513-8. doi: 10.3109/13880209.2012.688057. Epub 2012 Sep 14.

Aisen PS, Schneider LS, Sano M, Diaz-Arrastia R, van Dyck CH, Weiner MF, Bottiglieri T, Jin S, Stokes KT, Thomas RG, Thal LJ; Alzheimer Disease Cooperative Study.High-dose B vitamin supplementation and cognitive decline in Alzheimer disease: a randomized controlled trial. JAMA. 2008 Oct 15;300(15):1774-83. doi: 10.1001/jama.300.15.1774.

Chatterjee N, Rastogi RP, Dhar ML (1963) Chemical examination of Bacopa munniera Wettst.: Part I – Isolation of chemical constituents. Indian J Chem, : 212–215.

Committee for Proprietary Medicinal Products (CPMP). Points to consider on multiplicity issues in clinical trials. EMEA 2002.

De Rijk MC1, Breteler MM, den Breeijen JH, Launer LJ, Grobbee DE, van der Meche FG, Hofman A "Dietary antioxidants and Parkinson disease. The Rotterdam Study. Arch Neurol. 1997 Jun;54(6):762-5.

Deschamps V, Barberger-Gateau P, Peuchant E, Orgogozo JM. Nutritional factors in cerebral aging and dementia: epidemiological arguments for a role of oxidative stress. Neuroepidemiology. 2001 Feb;20(1):7-15.

Di Matteo V, Pierucci M, Di Giovanni G, Dragani LK, Murzilli S, Poggi A, Esposito E. Intake of tomatoenriched diet protects from 6-hydroxydopamine-induced degeneration of rat nigral dopaminergic neurons J Neural Transm Suppl. 2009;(73):333-41.

Engelhart M, Geerlings MI, Ruitenberg A, Van Swieten JC, Hofman A, Witteman JC, Breteler MM., Diet and risk of dementia: Does fat matter?: The Rotterdam Study. Neurology. 2002 Dec 24;59(12):1915-21.

Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM Astaxanthin: a review of its chemistry and applications. Crit Rev Food Sci Nutr. 2006;46(2):185-96.

Higuera-Ciapara I1, Felix-Valenzuela L, Goycoolea FM. Crit Rev Food Sci Nutr. 2006;46(2):185-96 Astaxanthin: a review of its chemistry and applications.

Jenner P, Olanow CW "Oxidative stress and the pathogenesis of Parkinson's disease Neurology" 1996 Dec;47(6 Suppl 3):S161-70.Karppi J, Laukkanen JA, Sivenius J, Ronkainen K, Kurl S. Neurology. 2012 Oct 9;79(15):1540-7. doi:

10.1212/WNL.0b013e31826e26a6 Serum lycopene decreases the risk of stroke in men: a population-based follow-up study.

18. Public Protocol Approval Page

Study Title: "Effect of an antioxidants mix on cognitive performance and well being: The Bacopa, Licopene, Astaxantina, Vitamin B12" – BLAtwelve study

Code: MEIF/17/BAC-COG/001

EUDRA-CT number: not applicable

The signers confirm that they have read and approved the protocol

Study Medical Expert: *(name in capital letters)* _____

Signature & Date: _____ _____ / _____ / _____

Coordinating Investigator: (if applicable) *(name in capital letters)* _____

Signature & Date: _____ _____ / _____ / _____

Statistician: *(name in capital letters)* _____

Signature & Date: _____ _____ / _____ / _____

Project Leader CRO (if applicable) *(name in capital letters)* _____

Signature & Date: _____ _____ / _____ / _____

Corporate Medical Director/International Scientific CU Manager: _____

Signature & Date: _____ _____ / _____ / _____

19. Investigator's approval Page (original to be kept in the Trial Center File)

I understand that all information concerning the product Bacopa, Licopene, Astaxantina, Vitamin B12 supplied by A. Menarini Industrie Farmaceutiche Riunite S.r.l., Sponsor of the study, in connection with this study protocol are confidential information. This information include: Protocol, Investigator's Brochure, Case Report Form, Other documents.

I understand that any change in this study protocol must be approved in writing by the Sponsor, the Co-ordinating Investigator and the Ethics Committee before implementation, except where necessary to eliminate apparent immediate hazard to subjects.

I confirm that I will conduct the study according to this protocol (except when mutually agreed to in writing with the Sponsor), the Good Clinical Practice (GCP), the Declaration of Helsinki and laws and regulations in the Country where the study is to be conducted.

I confirm that I will record and report all adverse events occurring during the study, according to this protocol.

I confirm that I am informed about the need of data records retention, according to current regulations and that no data can be destroyed without the written consent of the Sponsor.

I confirm that I will transfer adequate ownership of my responsibilities for the trial and will inform the Sponsor, in case I retire from my PI role.

I confirm that in case the Trial Center File is stolen or anyhow damaged, I will promptly inform the Sponsor and declare it to the Competent Authorities.

Principal Investigator: _____

Signature & Date: _____

20. APPENDICES

20.1 Appendix 1: Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964
and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

2. Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.

6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.

10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.

12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.

13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

14. Physicians who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

Risks, Burdens and Benefits

16. In medical practice and in medical research, most interventions involve risks and burdens.

Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.

Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.

When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

Vulnerable Groups and Individuals

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.

All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

Scientific Requirements and Research Protocols

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.

The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.

In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

Research Ethics Committees

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.

The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study's findings and conclusions.

Privacy and Confidentiality

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

Informed Consent

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.

After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

All medical research subjects should be given the option of being informed about the general outcome and results of the study.

27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.

28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.

29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.

30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.

31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.

32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

Use of Placebo

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:

Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or

Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.

Extreme care must be taken to avoid abuse of this option.

Post-Trial Provisions

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

Research Registration and Publication and Dissemination of Results

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.

36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

Unproven Interventions in Clinical Practice

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.