PROTOCOL

Effect of High-Dose-Vitamin-B Multivitamin Supplement on Neural Connectivity and Oxidative Metabolism in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled, Phase I Clinical Trial

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Protocol Number: Exec B
Version: 1.4
Date: 23 December 2020

Statement of Compliance

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP). The protocol, informed consent form(s), recruitment materials and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. A determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form. The principal investigator will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor, funding agency and documented approval from the IRB, except where necessary to eliminate an immediate hazard(s) to the trial participants. All personnel involved in the conduct of this study have completed the ICH GCP Training.

Contents

ADMINISTRATIVE INFORMATION

1. Title

Effect of High-Dose-Vitamin-B Multivitamin Supplement on Neural Connectivity and Oxidative Metabolism in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled, Phase I Clinical Trial

2. Trial Registration

2.1 Registry

To be updated later.

2.2 Data set

Data Category	**Information**
Source(s) of monetary or material support	Blackmores Institute, Australia
Primary sponsor	Taylor's University, Malaysia
Secondary sponsor(s)	Blackmores Institute, Australia
Contact for public queries	Assoc. Prof Dr Yeong Chai Hong Phone: +60 16 701 6875 Email: chaihong.yeong@taylors.edu.my
Contact for scientific queries	Assoc. Prof Dr Yeong Chai Hong Phone: +60 16 701 6875 Email: chaihong.yeong@taylors.edu.my
Public title	Vitamin B Multivitamin Supplement on Neural Connectivity and Oxidative Metabolism
Scientific title	Effect of High-Dose-Vitamin-B Multivitamin Supplement on Neural Connectivity and Oxidative Metabolism in Healthy Adults: A Randomised, Double-Blind, Placebo-Controlled, Phase I Clinical Trial
Countries of recruitment	Malaysia
Health condition(s) or problem(s) studied	Neural Connectivity and Oxidative Metabolism
Intervention(s)	Oral tablet

Data Category	Information
Key inclusion and exclusion criteria	Inclusion Criteria: • Healthy non-smoking males and females aged between 30 and 55 years • Not heavy consumers of alcohol (i.e., females consumed < 14 standard drinks per week, males consumed < 28 standard drinks per week) • No history of psychiatric disorders including clinical depression, anxiety or epilepsy • No history of / do not currently suffer from heart disease or high blood pressure or diabetes • Free from cognitive and memory impairment and does not suffer from any neurological disorders • Not taking any form of vitamin, mineral, herbal supplement, medications or illicit drugs which might reasonably be expected to interfere with cognition or mood for 4 weeks prior to (and duration of) the study such as multivitamins, B vitamins, *Ginkgo biloba*, antioxidants or other supplements • Not taking any form of medication within 5 days of admission (except for prophylactic antibiotics, or other routine medications to treat benign conditions, such as antibiotics to treat acne) and agree not to take any medication throughout the study • No clinically relevant abnormalities in their medical history that would render them ineligible for MRI • Not pregnant or possibility of being pregnant Exclusion Criteria: • Cigarette smoker • Current heavy regular use of alcohol exceeding 14 standard drinks per week for women and 28 standard drinks per week for men) • Gluten intolerance • Having claustrophobia (fear of constrained space) • Diagnosis of Type 1 or Type 2 diabetes • History of anxiety, depression, psychiatric disorders or epilepsy • History of / currently suffers from heart disease or high blood pressure • History of head injury/stroke • Evidence or history of any clinically significant (in the judgment of the investigator) renal, endocrine, pulmonary, gastrointestinal, cardiovascular, psychiatric, neurological, within the last 5 years • Clinically relevant abnormalities in their medical history that would render them ineligible for MRI • Currently taking Warfarin • Having metallic implants and other abnormalities in their medical history that would render them ineligible for MRI

Data Category	Information
Study type	Mode of action and safety profile
Date of first enrolment	July, 2020
Target sample size	90
Primary outcome(s)	• To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural connectivity of default mode network using resting-state functional magnetic resonance imaging (rs-fMRI) and diffusion tensor imaging (DTI) in healthy adults. • To study the safety profile of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract in healthy adults through blood plasma analysis.
Secondary outcome(s)	• To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural metabolites for oxidative stress in healthy adults using proton magnetic resonance spectroscopy (1H-MRS). • To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on plasma biomarkers for oxidative stress in healthy adults through analysis of plasma biomarkers. • To differentiate the effects of *Passiflora Incarnata* herbal extract on neural connectivity of default mode network and oxidative metabolism in healthy adults.

Approval:

23 December 2020

Associate Professor Dr. Yeong Chai Hong
School of Medicine, Faculty of Health and Medical Sciences,
Taylor's University, Selangor, Malaysia

Date

3. Protocol Version

Issue Date:	23 December 2020
Protocol version amendment number:	1.4
Author(s):	Associate Prof Dr Yeong Chai Hong Associate Prof Dr Anjan Kumar Das Prof Dr Rusli Nordin Dr Wong Yin How Associate Prof Dr Karuthan Chinna Mr Selvaraja Seerangam Prof Dr Norlisah Ramli Dr Azlan Che Ahmad Dr Tan Li Kuo

Revision Chronology:

Date of change	Summary of changes

4. Funding

Vitamin-B Multivitamin supplement and the matching placebo will be manufactured by Blackmores Ltd., Australia. Blackmores Institute is funding the run-in costs, trial intervention and placebo for the entire trial study. The design, management, analysis and reporting of the study are entirely independent of the manufacturer and funding agency.

5. Roles and Responsibilities

5.1 Contributorship

Researchers Name & Area of Expertise	Summary of Contribution
Associate Prof Dr Yeong Chai Hong **Medical Physicist - Radiology** School of Medicine, Faculty of Health and Medical Sciences, Taylor's University	Principal investigator. Initiates the study design, contributes to the refinement of the study protocol, execute the study, and the grant holder.
Prof Dr Rusli Nordin **Clinician - Public Health** School of Medicine, Faculty of Health and Medical Sciences, Taylor's University	Co-investigator. Initiates the study design, contributes to the refinement of the study protocol, recruitment of participants, taking consent, and provide professional advice in the aspect of public health.
Associate Prof Dr Anjan Kumar Das **Clinician - Clinical Trials** School of Medicine, Faculty of Health and Medical Sciences, Taylor's University	Co-Investigator. Initiates the study design, contributes to the refinement of the study protocol, recruitment of participants, taking consent, and provides professional advice on clinical ethics.
Dr Wong Yin How **Biochemistry** School of Medicine, Faculty of Health and Medical Sciences, Taylor's University	Co-investigator. Initiates the study design, contributes to the preparation of the study protocol, and assist in study implementation.
Associate Prof Dr Karuthan Chinna **Statistician - Biostatistics** School of Medicine, Faculty of Health and Medical Sciences, Taylor's University	Co-investigator. Provides statistical expertise in clinical trial design and conducts the primary statistical analysis.
Mr Selvaraja Seerangam **Pharmaceutical Regulatory** School of Pharmacy, Faculty of Health and Medical Sciences, Taylor's University	Co-investigator. Provides professional advice on ethical and licensing issues related to pharmaceutical aspects.
Prof Dr Norlisah Ramli **Clinician - Neuroradiologist** Department of Biomedical Imaging, Faculty Medicine, University of Malaya	Co-investigator. Conceives the study, contributes to the refinement of the study protocol, taking consent and interpreting the MRI data.
Dr Azlan Che Ahmad **MRI Physicist** Department of Biomedical Imaging, Faculty Medicine, University of Malaya	Co-investigator. Provides technical advice on MRI and MRS analysis, contributes to the refinement of scanning protocols, and provides professional advice on MRI safety.
Dr Tan Li Kuo **MRI Physicist and Biomedical Engineer** Department of Biomedical Imaging, Faculty Medicine, University of Malaya	Co-investigator. Contributes to the data analysis of the structural and functional MRI data.

5.2 Sponsor Contact Information

Study Sponsor	Taylor's University
Sponsor's Reference number (if applicable)	N/A
Contact name	Assoc. Prof. Dr. Yeong Chai Hong
Address	School of Medicine, Faculty of Health and Medical Sciences, Taylor's University, Lakeside Campus, No. 1, Jalan Taylor's, 47500 Subang Jaya, Selangor Darul Ehsan, Malaysia
Telephone	+60 16 701 6875
Email	Chaihong.yeong@taylors.edu.my

5.3 Sponsor and Funder

This funding agency had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

5.4 Study Committees

Principal Investigator and Research team:

- Associate Prof Dr Yeong Chai Hong
- Prof Dr Rusli Nordin
- Associate Prof Dr Anjan Kumar Das
- Dr Wong Yin How
- Associate Prof Dr Karuthan Chinna
- Mr Selvaraja Seerangam
- Prof Dr Norlisah Ramli
- Dr Azlan Che Ahmad
- Dr Tan Li Kuo

Lead Investigator:

- Associate Prof Dr Yeong Chai Hong

Clinical Co-Investigators:

- Prof Dr Norlisah Ramli (Neuroradiologist)
- Prof Dr Rusli Nordin (Public Health)
- Associate Prof Dr Anjan Kumar Das (Clinical Trials)

6. Study Introduction

6.1 Background and Rationale

Micronutrients, such as B vitamins, are essential for the optimal performance of human physiological processes [1, 2]. The B Vitamins is a group of water soluble organic molecules that function as co-factors in a wide range of cellular anabolic- and catabolic metabolisms. B vitamins are typically synthesised by plants with the exception to vitamin B12, which is synthesised by bacteria. In plants and animals that consume them, the B vitamins support energy metabolism and drive synthesis of DNA/RNA, antioxidants and neurotransmitters [3]. In addition, they have both direct (e.g. receptor binding, membrane ion pump function) and indirect effects (e.g. cerebral blood supply) on the brain functions [4]. Dietary status of certain B vitamins (e.g., folate, vitamins B6 and B12) has been positively associated with cognition, such as information processing, recall and verbal ability in healthy women of various ages [4]. Deficiency of these vitamins has been shown to results in fatigue, anxiety, irritability, sleeplessness and impaired memory and ability to concentrate [5].

Beneficial effects of B vitamins on cognitive functions/performance have been closely related to the function of B vitamins in different cellular metabolism such as synthesis and integrity of phospholipids, DNA and proteins [6]. Several B vitamins such as folate, B6 and B12, are also known to involve in the regulation of healthy levels of amino acid homocysteine, maintenance of cardiovascular and neural health and are vital for energy metabolism [10-12]. In addition, these B vitamins are essential for the methylation of homocysteine to methionine in the central nervous system. The methionine has a crucial role in biological processes for DNA synthesis, repair and other methylation reactions through one-carbon metabolism [7]. An elevated level of homocysteine would increase the likelihood for oxidative stress, leading to mitochondrial membrane damage and DNA strand breakage [13, 14]. Although the role of homocysteine in disease pathogenesis remains unclear, it may play a direct role in the disease process or simply be a marker of folate, B6 and or B12 vitamin deficiency. Previous research has identified that chronic stress depletes vitamin B6 [10] while supplementation with B6 vitamins could be a therapeutic strategy in reducing stress [11]. In addition, the B vitamins also involved in folate metabolism, which is closely correlate with mood and cognitive performance [7]. Vitamin B supplementation has also been shown to increase oxidative metabolism and thus reduces the neural inflammation and oxidative stress [19].

Looking at the pivotal role of B vitamins in cellular functions and thus the brain functions, manipulations of micronutrients such as B vitamins via multivitamin and multimineral supplementation may impact neurocognitive function [3]. The multivitamin and multimineral supplementation has been shown to enhance cognitive functions in the case of marginal and more severe deficiency [8, 9]. Previous study has suggested the reduction in fatigue and improve cognitive function following a 9-weeks supplementation [10] while another study indicates improved cognitive function in males but not women after 16-week supplementation [11]. Surprisingly, acute multivitamin supplementation has also produce positive effect in contentment and cognitive task performance in adults [2]. In addition, multivitamin supplementation for 12 weeks in children has improved cognitive performance [12]. These studies suggested improved mood and cognitive functions/performance through the supplementation even in the absence of vitamin deficiency by improving brain health.

In view of the above, investigation of the effect of a multivitamin and multimineral supplementation with high dose of B vitamins on the brain function, cognitive performance and

oxidative metabolism are warranted. In this proposed study, neural connectivity and cognitive performance after a 6-month multivitamins and multi-minerals supplementation (Executive B Formula from Blackmores) will be studied using neuroimaging techniques, i.e. structural and functional magnetic resonance imaging (fMRI). This technique has been used in a few studies to gather behavioural data and sample brain activity to evaluate the effect of micronutrients on brain function [1, 2, 9]. On the other hand, the effect of the supplementation will be investigated by determining the level of neural metabolites and plasma biomarkers for oxidative metabolism and neural inflammation. The concentration of several low molecular weight neural biomarkers of oxidative metabolism including total NAA (NAA + NAA-glutamate), total creatine (creatine + phosphocreatine), and total choline (phosphocholine + glycerylphosphorylcholine) will be assessed using proton magnetic resonance spectroscopy (1H-MRS). While the plasma biomarkers for oxidative metabolism such folate, B6, B16 and homocysteine will also be determined.

Passiflora incarnata (Passion flower) is a woody, hairy, climbing vine and is reputed to have anxiolytic property, and has been used widely as an ingredient of herbal remedies. It has been traditionally used in Western European traditional medicine to relieve stress and anxiety. The commission E has approved the internal use of passion flower for nervous restlessness, and the British Herbal Compendium indicates its use for sleep disorders, restlessness, nervous stress and anxiety [13-15]. Scientific literature indicates that B-vitamins may have similar effect [16]. Recent study on the formula that contained both B-Vitamins and Passion flower indicated that such effect may have been achieved through activation of brain centers responsible for pleasure, satisfaction and motivation [17-19]. Hence, this study is also taken to investigate whether activation of brain centers responsible for pleasure and motivation may primarily be attributed to the nutritional formula containing multivitamin-B or whether the inclusion of Passiflora Incarnata is providing additional/amplified/different effects/benefits over and above the basic nutritional formula containing multivitamin-B alone. The trial will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s).

6.2 Potential Risks & Benefits

6.2.1 Known Potential Benefits

The B vitamins has a pivotal role in cellular functions and thus the brain functions, would bring positive impact on neurocognitive function [3]. The multivitamin and multimineral supplementation has been shown to enhance cognitive functions in the case of marginal and more severe deficiency [8, 9]. Previous study [10] has reported reduction in fatigue and improve cognitive function following a 9-week supplementation while another study [11] reported improved cognitive function in men but not women after 16-week supplementation. In addition, acute multivitamin supplementation has also been reported in producing positive effect in contentment and cognitive task performance in adults [2], while multivitamin supplementation for 12-week in children has improved cognitive performance [12]. These studies suggested improved mood and cognitive functions/performance through the supplementation even in the absence of vitamin deficiency by improving brain health.

The cellular functions of the different composition of the study products are given in following Table 1.

Table 1: Cellular functions of the different composition of the Executive B Stress Formula 1 and 2.

Composition	Alternative Name	Cellular Functions
Thiamine hydrochloride	Vitamin B1 (Thiamine)	Thiamine is a coenzyme in the catabolism of sugars and amino acids. Thiamine plays a central role in the release of energy from carbohydrates.
Riboflavin	Vitamin B2	Riboflavin is a water-soluble vitamin involved in ATP production and the metabolism of many of the other B group vitamins.
Nicotinamide	Vitamin B3	Nicotinamide is a water-soluble nutrient involved in energy production and carbohydrate metabolism.
Calcium pantothenate	Vitamin B5 (Pantothenic acid)	Pantothenic acid (vitamin B5) is involved in the metaboloism of fats and carbohydrates for energy production.
Pyridoxine hydrochloride	Equivalent to Pyridoxine (Vitamin B6)	Pyridoxine hydrochloride (vitamin B6) is a water-soluble nutrient involved in the production of proteins, neurotransmitters and haemoblobin.
Biotin	Vitamin B7	Biotin plays a key role in the metabolism of lipids, proteins and carbohydrates.
Folic Acid	Vitamin B9, Folate	Folate acts as a co-enzyme in the form of tetrahydrofolate (THF), which is involved in the transfer of single-carbon units in the metabolism of nucleic acids and amino acids. It is also involved in the activation of vitamin B12 into its active form.
Vitamin B12	Cobalamin	Cyanocobalamin is an essential water-soluble nutrient needed for protein and DNA synthesis, folate metabolism, and red blood cell production.
Ascorbic acid	Vitamin C	Essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters.
D-Alpha tocopheryl acid succinate	Vitamin E	Natural tocopherol and one of the most potent antioxidant tocopherols. It exhibits antioxidant activity by virtue of the phenolic hydrogen on the 2H-1-benzopyran-6-ol nucleus.
Magnesium phosphate	Dietary source of magnesium	It helps maintain energy, combat fatigue, build and maintain bones and teeth, contributes to the health of nerve, muscle and cell membranes, and contributes to normal muscle function, including the

		heart, helps with protein manufacture.
Potassium sulfate	Dietary source of potassium	Plays a role in many body functions including transmission of nerve signals, muscle contractions, fluid balance, and various chemical reactions.
Choline bitartrate	Choline	Structural integrity and signalling roles for cell membranes, cholinergic neurotransmission (acetylcholine synthesis).
Inositol	myo-inositol, often referred to as vitamin B8	Mediates cell signal transduction in response to a variety of hormones, neurotransmitters and growth factors and participates in osmoregulation.
Zinc oxide	Dietary source of zinc	Plays a role in cell division, cell growth, wound healing, and the breakdown of carbohydrates.
Avena sativa extract	Oats	Normally consumed as whole-grain cereal and contain small amounts of free phenolic acids, vanillic acids, flavonoids and avenanthramides (AVs), which are hydroxycinnamoylanthranilate alkaloids unique to oats. AVs are bioavailable and thought to be antiatherogenic due to their antioxidant, antiproliferative and anti-inflammatory activities, as such, they could be acting in synergy with other antioxidants in the formulation. Traditionally, the extract is used to relief of mild symptoms of mental stress and to aid sleep.
Passiflora incarnata extract	Passion flower	Extensively as an anxiolytic and sedative, with its central nervous system depressant effects being attributed to harmala alkaloids and flavonoid content. Its anxiolytic effect may have contributed to the reduction in negative mood states. Traditional medicinal use of *Passiflora incarnata* for the relief of mild symptoms of mental stress and to aid sleep is well documented in a number of recognised handbooks. The pharmacodynamic studies in animals and the clinical studies may be seen as supportive to the plausibility of the traditional use of *Passiflora incarnata*.

6.2.2 *Known* Potential Risks

Long-term use of broad-spectrum multivitamin and multimineral supplements has not been associated with any major safety concerns as long as they do not exceed the upper limit (UL) recommended by the authorities [20, 21]. A systematic review of 15 multivitamin and

multimineral supplement studies reported that only mild gastrointestinal adverse events were observed during the studies [21]. In addition, use of multivitamin and multimineral supplement has been associated with a modest increase in skin rashes, as well as some inconsistent effects on minor bleeding that were considered to be more likely a function of chance than effect [22]. However, in another randomized, controlled phase II study involving multivitamin and multimineral supplement, no significant effects on gastrointestinal side effects, fatigue, drowsiness, skin discoloration or migraine was observed [22]. A previous study performed on human with a similar dosage has not reported any adverse events associated with the multimineral and multivitamins supplementation. Furthermore, the clinical trials performed in Australia using the Executive B Stress Formula with a similar composition of the investigator product of this study [17-19] have not reported any adverse event during the study. The value of the dosage of different minerals and vitamins along with their recommended daily allowance (RDA), tolerable upper intake levels (UL) and adverse effects of excessive consumption are given in Table 2. All the compositions in the investigator products do not exceed the UL except Nicotinamide (Vitamin B3). The potential adverse effects of excessive Nicotinamide are flushing and gastrointestinal distress. Nevertheless, Nicotinamide is water-soluble and our body does not store Nicotinamide whereby the excessive Nicotinamide will be excreted from the body through urine.

Table 2: The recommended daily allowance (RDA), tolerable upper intake levels (UL), adverse effects of excessive consumption for the vitamins and minerals presence in Executive B Stress Formula 1 and 2.

Composition, unit	RDA	Tolerable Upper Intake Levels (UL)	Executive B Stress Formula 1	Executive B Stress Formula 2	Adverse Effects of Excessive Consumption
Vitamin B1, mg	1.2	ND	50	50	No adverse effects associated with Vitamin B1 from food or supplements have been reported.
Vitamin B2, mg	1.3	ND	10	10	No adverse effects associated with Vitamin B2 consumption from food or supplements have been reported.
Nicotinamide, mg	16	35	100	100	There is no evidence of adverse effects from the consumption of naturally occurring niacin in foods. Adverse effects from niacin containing supplements may include flushing and gastrointestinal distress.
Pantothenic acid, mg	5	ND	68.71	68.71	No adverse effects associated with pantothenic acid from food or supplements have been reported.
Vitamin B6, mg	1.3	100	21	21	No adverse effects associated with Vitamin B6 from food or supplements have been reported.
Folic acid, µg	400	1000	200	200	Masks neurological complication in people with vitamin B12 deficiency. No adverse effects associated with folate from food or supplements have been reported.
Vitamin B12, µg	2.4	ND	30	30	No adverse effects associated with Vitamin B12 consumption from food or supplements have been reported.
Choline, mg	550	3500	25	25	Fishy body odor, sweating, salivation, hypotension, hepatotoxicity.
Vitamin C, mg	900	2000	250	250	Gastrointestinal disturbances, kidney stones, excess iron absorption.

Vitamin E, mg	15	1000	25.94	25.94	There is no evidence of adverse effects from the consumption of vitamin E naturally occurring in foods. Adverse effects from vitamin E containing supplements may include hemorrhagic toxicity.
Biotin, µg	30	ND	50	50	No adverse effects of biotin in humans or animals were found.
Magnesium, mg	400	350	52.50	52.50	There is no evidence of adverse effects from the consumption of naturally occurring magnesium in foods. Adverse effects from magnesium containing supplements may include osmotic diarrhea. The UL for magnesium represents intake from a pharmacological agent only and does not include intake from food and water.
Potassium, mg	4700	No	33.7	33.7	None documented from food alone; however, potassium from supplements or salt substitutes can result in hyperkalemia and possibly sudden death if excess is consumed by individuals with chronic renal insufficiency (kidney disease) or diabetes.
Inotisol, mg	ND since it is not considered an essential nutrient.	ND	25	25	Mild side effects have been reported with doses of 12 grams per day or higher. These include nausea, gas, difficulty sleeping, headache, dizziness and tiredness [23].
Zinc, mg	9.4	40	15	15	Reduced copper status.

RDA= Recommended Daily Allowance; ND = Not Determine

A Tolerable Upper Intake Level (UL) is the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects to almost all individuals in the general population. Unless otherwise specified, the UL represents total intake from food, water, and supplements. Due to a lack of suitable data, ULs could not be established for vitamin K, thiamin, riboflavin, vitamin B12, pantothenic acid, biotin, and carotenoids.

SOURCES: Dietary Reference Intakes for Calcium, Phosphorous, Magnesium, Vitamin D, and Fluoride (1997); Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline (1998); Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids (2000); Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc (2001); Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate (2005); and Dietary Reference Intakes for Calcium and Vitamin D (2011). These reports may be accessed via www.nap.edu.

6.3 Explanation for Choice of Comparators

The matching placebo will be produced using the same base ingredients as that found in active intervention. The size, shape, type of coating and colour of the placebo will be matched to that of active intervention. The placebo will contain glucose and trace quantities of Riboflavin (B2) to be matched for colour and taste and to provide a similar urine colouration effect.

6. Objectives

7.1 Research Aim and Hypothesis

The general aim of the proposed study is to investigate the effect of high-dose-B-vitamin multivitamin supplementation (Executive B Stress Formula) with and without *Passiflora Incarnata* herbal extract on neural connectivity of the default mode network and oxidative metabolism in healthy adults. More specifically, a 6-month supplementation of high-dose-B-vitamin multivitamin is expected to improve neural connectivity of default mode network and oxidative metabolism in healthy adults.

7.2 Study Objectives

Primary Objective:

To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural connectivity of default mode network in healthy adults using resting state fMRI and Diffusion Tensor Imaging (DTI). To the study the safety profile of high-dose-B-vitamin multivitamin supplement with and without Passiflora Incarnata herbal extract in healthy adults through plasma analysis.

Secondary Objectives:

- To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural metabolites for oxidative stress in healthy adults.
- To study the effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on plasma biomarkers for oxidative stress in healthy adults.
- To differentiate the effect of *Passiflora Incarnata* herbal extract on neural connectivity of default mode network and oxidative stress metabolism in healthy adults.

7. Study Design

This is a single-site, double-blind, randomised, parallel, clinical trial utilising active and placebo arms to assess the effect of high-dose B-vitamins multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural connectivity of default mode network and oxidative metabolism in healthy adults. The study will be conducted between July, 2020 to June, 2021 at the Taylor's University Lakeside Campus, Subang Jaya, Malaysia and the 3T MRI Research Centre, University of Malaya, Kuala Lumpur, Malaysia. The study will include three arms, namely, active intervention A, active intervention B and placebo comparator group with 30 subjects in each arm. Randomization will be performed with a 1:1:1 allocation between the active intervention and placebo groups. The composition of the Executive B Stress Formula 1 (active intervention 1) and Executive B Stress Formula 2 (active intervention 2) are given in Table 3.

Active intervention A:	Executive B Stress Formula 1 - with *Passiflora Incarnata* herbal extract
Active intervention B:	Executive B Stress Formula 2 - without *Passiflora Incarnata* herbal extract
Placebo:	Placebo will contains glucose and trace quantities of Riboflavin (B2) to match the colour and taste of the active ingredient

Table 3. Chemical composition of the study products

Actives		**Executive B Stress Formula 1**	**Executive B Stress Formula 2**
Thiamine hydrochloride	mg	56.06	56.06
Equiv. to Thiamine	mg	50	50
Riboflavin	mg	10	10
Nicotinamide	mg	100	100
Pyridoxine hydrochloride DC 97%	mg	26.28	26.28
Equiv. to Pyridoxine	mg	21	21
Ascorbic acid DC 97%	mg	257.73	257.73
Equiv. Ascorbic acid	mg	250	250
D-Alpha tocopheryl acid succinate DC	mg	25.94	25.94
Equiv. to Vitamin E	IU	30.00	30.00
Calcium pantothenate	mg	75.00	75.00
Equiv. to Pantothenic acid	mg	68.71	68.71
Magnesium phosphate	mg	254.15	254.15
Equiv. to Magnesium	mg	52.50	52.50
Potassium sulfate	mg	75.15	75.15
Equiv. to Potassium	mg	33.70	33.70
Vitamin B12 1% in DCP	mg	3.00	3.00
Equiv. Vitamin B12 (Cyanocobalamin)	mcg	30.00	30.00
Biotin	mcg	50.00	50.00
Folic acid	mcg	200.00	200.00
Avena sativa (Oat) seed - extract dry concentrate 10:1, 100% W *Excipient: Maltodextrin 35%*	mg	25.00	25.00
Equiv. to Avena sativa (Oat) seed - dry	mg	250.00	250.00
Passiflora incarnata (Passionflower) herb - extract dry concentrate 5:1; 60% E:W *Excipients: Maltodextrin 18%; Silica colloidal anhydrous 2%*	mg	20.00	N/A
Equiv. to Passiflora incarnata (Passionflower) herb - dry	mg	100.00	N/A

Choline bitartrate	mg	25.00	25.00
Inositol	mg	25.00	25.00
Zinc oxide	mg	19.23	19.23
Equiv. to Zinc	mg	15.00	15.00
Excipients			
Microcrystalline cellulose	mg	139.19	139.19
Croscarmellose sodium	mg	50.00	50.00
Ethylcellulose	mg	12.00	12.00
Silica colloidal anhydrous	mg	25.00	25.00
Magnesium stearate	mg	15.00	15.00
Lecithin	mg	50.00	50.00
Opadry complete film coating system - brown 03M665005 (hypromellose, caramel, glycerin)	mg	42.00	42.00
Carnauba wax	mg	q.s.	q.s.

METHODS: PARTICIPANTS, INTERVENTIONS, OUTCOMES

8. Study Setting

The study will be conducted between July, 2020 to June, 2021 at the Taylor's University Lakeside Campus, Subang Jaya, Malaysia and the 3T MRI Research Centre, University of Malaya, Kuala Lumpur, Malaysia.

9. Eligibility Criteria

10.1 Inclusion Criteria

In order to participate in this study, an individual must meets the following inclusion criteria:

- Non-smoking males and females aged between 30 and 55 years
- Not heavy consumers of alcohol (i.e., females consumed < 14 standard drinks per week, males consumed < 28 standard drinks per week)
- No history of psychiatric disorders including clinical depression, anxiety or epilepsy
- No history of / do not currently suffer from heart disease or high blood pressure or diabetes.
- No history from cognitive and memory impairment and does not suffer from any neurological disorders
- Not taking any form of vitamin, mineral, herbal supplement, medications or illicit drugs which might reasonably be expected to interfere with cognition or mood such as multivitamins, B vitamins, ginkgo biloba, antioxidants or other supplements for 4 weeks prior to and during the study period.
- Not taking any form of medication within 5 days of admission (except for prophylactic antibiotics, or other routine medications to treat benign conditions, such as antibiotics to treat acne) and agree not to take any medication throughout the study
- No clinically relevant abnormalities in their medical history that would render them ineligible for MRI
- Not pregnant or possibility of being pregnant

10.2 Exclusion Criteria

Participants presenting with any of the following will not be included in the trial:

- Cigarette smoker
- Current heavy regular use of alcohol exceeding 14 standard drinks per week for women and 28 standard drinks per week for men)
- Gluten intolerance
- Having claustrophobia (fear of constrained space)
- Diagnosis of Type 1 or Type 2 diabetes
- History of anxiety, depression, psychiatric disorders or epilepsy
- History of / currently suffers from heart disease or high blood pressure
- History of head injury/stroke
- Evidence or history of any clinically significant (in the judgment of the investigator) renal, endocrine, pulmonary, gastrointestinal, cardiovascular, psychiatric, neurological, within the last 5 years

- Clinically relevant abnormalities in their medical history that would render them ineligible for MRI
- Currently taking Warfarin
- Having metallic implants and other abnormalities in their medical history that would render them ineligible for MRI

10. Interventions

11.1 Interventions

Participants who have expressed interest in the study will be sent an information sheet outlining the details of the project. Phone / face-to-face screening will be carried out to evaluate the participant's eligibility in the study prior to scheduling a baseline visit. The participants will adhere to a total of 4 visits for baseline measurement, mid-intervention assessment and post-intervention assessment.

During baseline visit, inclusion and exclusion criteria will be reviewed followed by an initial health assessment including lifestyle questions (smoking, caffeine, alcohol intake etc.), current medications, medical history, current supplements, anthropometric measurements (weight and height), vital signs (heart rate and blood pressure) and fasting blood glucose level measurements. Once it has been determined that the participant is eligible for the study, he/she will be issued with a consent form, and required to sign the consent form and return this to the Investigator. A fasting blood sample will be collected from participants for plasma vitamin B12, folate and homocysteine measurements. Additionally, safety profiling will be measured through a full blood count and biochemical liver function tests. The resting state fMRI, DTI and 1H-MRS will also be performed during baseline visit. The resting state fMRI and DTI will be used to access the neural connectivity of default mode network of the brain while the 1H-MRS will be used to quantify low molecular weight metabolites including N-acetyl aspartate (NAA), choline-containing compounds (collectively referred to herein as "choline"), creatine (including phosphocreatine), in the posterior cingulate cortex (PCC) and left dorsolateral prefrontal cortex (DLPFC). The general intelligence (IQ) will be assessed using Wechsler abbreviated scale of intelligence (WASI) before the MRI scanning.

The participant will be then randomly assigned to active intervention A, active intervention B or placebo groups to receive oral tablets of either Executive B Stress Formula 1 or Executive B Stress Formula 2 or matching placebo. During the intervention, each participant will consume two tablets per day – one at breakfast and one at lunch – for the duration of 24 weeks. Each participant will receive sufficient tablets for 24 weeks, along with additional two week of tablets in case the post-intervention visit date was delayed. The formulation of the active intervention A, active intervention B and placebo is given in Table 1. The placebo tablets will contain glucose and trace amount of Riboflavin (B2) to match the colour and taste of active intervention and to provide a similar urine colouration effect.

Active intervention A:	Executive B Stress Formula 1 - with *Passiflora Incarnata* herbal extract
Active intervention B:	Executive B Stress Formula 2 - without *Passiflora Incarnata* herbal extract
Placebo:	Placebo will contains glucose and trace quantities of Riboflavin (B2) to match the colour and taste of the active ingredient

During the second visit (Day 42), vital signs and fasting blood samples will be collected for the plasma oxidative biomarkers quantification and blood safety profiling. The resting state fMRI, DTI and 1H-MRS will also be performed. In study visit 3 (Day 84), the resting state fMRI, DTI and 1H-MRS will be performed.

During the fourth visit (Day 168), vital signs and fasting blood samples will be collected for plasma biomarkers quantification and blood safety profiling. The rs-fMRI, DTI and 1H-MRS will also be performed. To ensure the compliance to the treatment schedule, the remaining tablets will be counted at the fourth study visit. Participants will also be asked to refrain from drinking alcohol for 24 hours and not to drink caffeine 12 hours prior to any study visits.

MRI Scanning

MRI will be performed using a 3-Tesla MRI system (Siemens MAGNETOM Prisma) with a 64-channel head coil at the UM MRI Research Centre, University of Malaya, Kuala Lumpur, Malaysia. Participants will be required to change into a hospital gown and to remove all metallic objects. A MRI safety checklist will be filled (with guidance from the technologists) and signed by the participants prior to enter to the MRI suite. The participants will be placed in the MRI scanner and provided with an emergency button. Participants will be asked to minimise head movement, which is aided by foam padding inserted around the participant's head and neck. There will be a build-in intercom to enable communication between the participants and the researcher while in the scanner. The MRI operator will be in radio communication with the participant throughout the scan, and will monitor the participant for any problems related to movement restrictions. Participants will be lying supine in the scanner throughout the whole MRI procedure.

T1-weighted MRI Procedure

T1-weighted images will be acquired using a magnetisation prepared rapid gradient echo (MPRage) pulse sequence (slice thickness = 1.0 mm, voxel resolution = 1.0 mm^3, TR = 1900 ms, TE = 2.52 ms, TI= 900 ms, bandwidth= 170 Hz/px, flip angle = 9°, field of view 350 × 263 × 350 mm).

rs-fMRI Procedure

rs-fMRI will be used to assess interactive brain networks in the passive state of rest [24]. The default mode network is part of the resting state network, which includes a specific set of brain regions (prefrontal cortex, posterior cingulate cortex, inferior parietal lobule, precuneus, hippocampal formation and the temporal cortex). The default mode network has been reported to be involved in important cognitive functions of internal mental activity such as emotional processing, personal introspection and autobiographical memories [25]. For the rs-fMRI data acquisition, a structural scan will be obtained in the first section where the participants will rest in the MR scanner for 3 minutes. During rs-fMRI data acquisition, participants will be instructed to remain still with their eyes open looking at a centrally placed white cross on a black background attached on the scanner. The resting state connectivity of the default mode network will be investigated using a seed identified by Suo et al., (2016) based in the posterior cingulate, using independent component analysis. Age, IQ, education years and gender will be entered as covariates in all analysis. The analysis will be performed using Statistical Parametric Mapping (SPM) software and NOODI method.

Diffusion Tensor Imaging (DTI)

A single shot spin-echo echo-planar imaging (EPI) will be used for DTI acquisition with the following reference parameters (recommended by Brain Canada Prisma Protocol, McGill Centre for Integrative Neuroscience, refer https://mcin-cnim.ca/research/neuroimaging-methods/acquisition-protocol/): 60 slices without a gap, FOV = 190 mm, phase FOV = 100%, slice thickness = 2 mm, base resolution = 96, phase resolution = 100 (voxel size 2 x 2 x 2 mm), phase partial Fourier = 6/8, TR = 9900 ms, TE = 102 ms, average = 1, b-value = 1400 s/mm^2, bandwidth = 1080 Hz, EPI factor = 96, echo spacing = 1 ms. Average ADC map, trace weighted map, FA map and tensor data will be created inline.

For DTI analysis, voxel-wise, whole brain tract-based spatial statistics (TBSS) will be carried out to process white matter tracts of interests on a group-wise basis (FSL v5.0.6 University of Oxford, UK) [26]. Standard pre-processing will be applied to all volumes, including eddy current correction, brain masking, and linear fitting of the diffusion tensors. Randomise, a sub-tool in TBSS will be used to carry out voxel-wise statistical analysis across the control and intervention groups. The analysis will be carried out with 10,000 permutations using threshold-free cluster enhancement with 2D optimization as correction for multiple comparison across voxels.

1H-MRS Biomarker Data Collection

The T1-weighted images will be used to position the PCC (20 × 20 × 20 mm) and left DLPFC (15 × 20 × 20 mm) voxels. For localised quantification of total NAA (NAA + NAA-glutamate), total creatine (creatine + phosphocreatine), and total choline (phosphocholine + glycerylphosphorylcholine), a PRESS sequence is employed with chemical shift selective (CHESS) [27] water suppression (TE = 30ms, TR = 2000ms, bandwidth = 1200Hz, 80 averages for PCC, 160 averages for DLPFC, acquisition time= 2 min 48 s). Eight and 16 spectral water averages (without water suppression) will be acquired with identical PRESS parameters and shim for the PCC and left DLPFC voxels, respectively.

Analyses will be conducted with TARQUIN version 4.3.7 which estimates signal amplitude using a non-negative least-squares projection of a parametrised basis set in the time-domain [28]. Eddy current correction will be applied. For both PCC and DLPFC voxels, data will be excluded if: a) signal to noise (SNR) is less than 10, b) the water line-width ($FWHM_{water}$) is greater than 12Hz, and/or c) visual inspection failed. All metabolite concentrations will be corrected for voxel CSF ratio and water concentration – due to water-scaling – using the following formula [29]:

$M_{corr}= M*(WM + GM + 1.55 \times CSF) / (WM + GM)$

where M_{corr} is the corrected metabolite concentration, M is the original metabolite concentration, and WM and GM are white matter and grey matter percentages, respectively.

General intelligence (IQ), Wechsler abbreviated scale of intelligence (WASI)

Participants will complete the vocabulary and matrix reasoning subsets of the Wechsler Abbreviated Scale of Intelligence (WASI). The vocabulary subset is a 42-item task, which requires the examinee to orally define words that are presented visually and orally. The matrix reasoning subtest shows a series of 35 incomplete grid patterns which the examinee is asked to complete by pointing to, or stating, the correct pattern from five possible choices. The WASI is a reliable measure of intelligence for use in clinical and research settings.

11.2 Modifications

The assigned study intervention could be modified or discontinued by principal investigator for various reasons, including harms and withdrawal of participant consent. The study intervention could be discontinued by principal investigator if any clinical adverse event (AE), laboratory abnormality or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.

11.3 Adherence

Face-to-face adherence reminder sessions will take place at the initial intervention dispensing and each study visit thereafter. This session will include:

- The importance of following study guidelines
- Instruction about taking the tablets twice a day after breakfast and lunch
- Notification that there will be a tablet count at the end of the study
- Reinforcement that study tablets may be the herbal supplements or placebo
- Importance of calling the clinic if experiencing problems possibly related to study intervention such as symptoms and lost tablets

Subsequent sessions will occur at the follow-up visits. Participants will be asked about any problems they are having after taking their study tablets. Participants will return the unused tablets and bottle during the sixth months' visit. Unused tablets will be counted and recorded on the appropriate case report form. Subjects will be monitored for compliance with the protocol by a combination of telephone and email communications. The doses taken will be assessed by number of returned tablets at completion of the study.

11.4 Prior and Concomitant Care

The administration any medications, herbal extracts, illicit drugs or vitamin and mineral supplements which might reasonably be expected to interfere with mood and cognition is not permitted for all participants 4 weeks prior to and duration of the study. A drastic change in the living lifestyle and diet are also not permitted during the 24-week trial study. If participants are taking any medications / treatments for benign conditions (e.g. allergy medication) during the study period, they will be asked to stop taking the medications 5 days before and during the post-intervention and follow-up visits. Criteria to be considered before implementing the above include the period of time and dosage of medication participant must take and the medication / treatment's capacity to interfere with study procedures, data integrity, or compromise participant safety. This will be determined by the principle investigator.

11. Outcomes

Primary Outcome Measures:

The effect of high-dose-B-vitamin multivitamin supplement with and without *Passiflora Incarnata* herbal extract on the connectivity of the default mode network will be assessed by rs-fMRI and DTI on specific brain regions. The safety end-point will be assessed through the vital signs measurements (heart rate and blood pressure) and clinical laboratory parameters (red and white blood cells, electrolyte, liver function, lipid profile). The safety evaluation will be performed during visit 2 (Day 42) and visit 4 (Day 168).

Secondary Outcome Measures:

The effect of high-dose-vitamin-B multivitamin supplement with and without *Passiflora Incarnata* herbal extract on neural metabolites and plasma biomarkers for oxidative stress will be assessed by 1H-MRS and blood plasma analysis for oxidative biomarkers.

Other Outcome Measures:

To differentiate the effect of *Passiflora Incarnata* herbal extract on neural connectivity of the default mode network and oxidative metabolism will be assessed by comparing the results of active intervention A and B groups.

12. Participant Timeline

TIMEPOINT**	***Visit 1 Day 0***	***Visit 2 Day 42***	***Visit 3 Day 84***	***Visit 4 Day 168***
PROTOCOL ACTIVITY				
Inclusion/exclusion	X			
Informed consent	X			
Medical / medication history	X			
Intercurrent medical issues review	X			
Review concomitant therapies	X			
Physical examination	X			
Vital signs (heart rate and blood pressure etc.)	X	X	X	X
Anthropomorphic measures	X			
Dispense product	X			
Pill count/compliance assessment				X
Fasting blood glucose level	X			
Randomization	X			
INTERVENTIONS				
Active intervention A	X	—	—	X
Active intervention B	X	—	—	X
Placebo	X	—	—	X
Magnetic Resonance Imaging				
Resting-state functional MRI	X	X	X	X
Diffusion Tensor Imaging (DTI)	X	X	X	X
Proton Magnetic Resonance Spectroscopy (1H-MRS)	X	X	X	X
Wechsler Abbreviated Scale Of Intelligence (WASI)	X			
HAEMATOLOGY AND SERUM CHEMISTRY*	X	X		X
COMPLETE CASE REPORT FORMS (CRFS)	X	X	X	X

* Red and white blood cells count, electrolyte, liver function and lipid profile. Plasma vitamin B12, vitamin B6, folate and homocysteine level determination.

Study Flow Chart

- Telephone / face-to-face screening for general inclusion and exclusion criteria.
- Invitation to the screening visit (Visit 1).

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Visit 1: Day 0 ± 7
Locations: Taylor's University, Life Care Diagnostics Medical Centre and University of Malaya Medical Centre*

- Initial health assessment including lifestyle questions, current medications, medical history, physical examination, vital signs, anthropometric measurements (weight and height), blood pressure and fasting blood glucose level.
- Confirm recruitment eligibility and obtain informed consent.
- Performed baseline measurements:
  - Collect fasting blood sample (Baseline data)
  - Resting state fMRI, DTI and 1H-MRS
- Wechsler Abbreviated Scale of Intelligence (WASI).

⇩

Confirm recruitment eligibility based on initial health assessment

Randomization

Active Intervention A (n = 30)	Active Intervention B (n = 30)	Placebo (n = 30)

Visit 2: Day 42 ± 7
Locations: Life Care Diagnostics Medical Centre and University of Malaya Medical Centre*

- Measure vital signs and collect fasting blood sample
- Resting state fMRI, DTI and 1H-MRS

⇩

Visit 3: Day 84 ± 7
Locations: Life Care Diagnostics Medical Centre and University of Malaya Medical Centre*

- Measure vital signs
- Resting state fMRI, DTI and 1H-MRS

⇩

Visit 4: Day 168 ± 7
Locations: Life Care Diagnostics Medical Centre and University of Malaya Medical Centre*

- Measure vital signs and collect fasting blood sample
- Resting state fMRI, DTI and 1H-MRS

** Life Care Diagnostic Medical Centre at No. 5, Jalan Kerinchi, Bangsar South, 59200 Kuala Lumpur, Wilayah Persekutuan Kuala Lumpur, Malaysia*

13. Sample Size

The sample size is determined referring to Thirion et al. [30] and Pajula & Tohka [31], which suggesting a minimum samples size of 25 should be included in functional neuroimaging studies in order to have sufficient reliability. In this study, to allow for 25% drop out, 30 participants will be recruited per arm, i.e., 90 in total in three arms.

14. Recruitment

The study population will include 90 Malaysian healthy adults, aged between 30–55 years. The study participants will be recruited through a trial recruitment database and public media. The enrolment period will extend over 4 months.

15. Allocation

16.1 Sequence Generation

Randomisation of the products will be performed independently of the investigators using sealed opaque and identical envelopes containing identity of either A, B or C group. The investigational products will be delivered to the investigators in trial product containers that are identical in function and appearance, marked as A, B or C. Once enrolled in the trial, participants will be randomly allocated to either A group (n = 30), B group (n = 30) or C group (n = 30). The participant is randomized into either the group A, B or C by choosing a closed envelope. Investigators will be blinded to the randomisation and therefore blinded to which subjects are allocated to the active and treatment arms.

16.2 Concealment Mechanism

Randomization will be performed independently of the investigators using sealed opaque, identical envelopes.

16.3 Implementation

The investigational products will be delivered to investigators in trial product containers that are identical in function and appearance, marked as A (n = 30), B (n = 30) and C (n = 30). The identity of A, B and C will only be disclosed to the investigators at the end of the study after the data analysis completed. Once enrolled in the trial, subjects will be randomly allocated to group A (n = 30), B (n = 30) or C (n = 30) and received the intervention labelled with labels A, B or C. Investigators are blinded to the randomisation and therefore blinded to which subjects are allocated to the active and treatment arms.

16. Blinding (Masking)

17.1 Blinding (Masking)

In this double-blinded trial study, principal investigator, research team and the participants will be blinded after assignment to interventions.

17.2 Emergency Unblinding

To maintain the overall quality and legitimacy of the study, code breaks occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the participants. If this occurs, the sponsor and the ethics committee will be informed.

METHODS: DATA COLLECTION, MANAGEMENT, ANALYSIS

17. Data Collection Methods

18.1 Data Collection Methods

The principal investigator and research team will be trained centrally in the study requirements, standardized measurement of weight, height, blood pressure, fasting blood glucose level, requirements for laboratory blood sample collection, counselling for adherence and the eliciting of information from study participants in a uniform reproducible manner. The data to be collected and the procedures to be conducted at each visit will be reviewed in detail. Each of the data collection forms and the nature of the required information will be discussed in detail on an item by item basis. Entering data forms, responding to data discrepancy queries and general information about obtaining research quality data will also be covered during the training session.

18.2 Retention

Once a participant is enrolled or randomized, the study site will make every reasonable effort to follow the participant for the entire study period through phone call and emails. A minimal travel allowance (amount as follow) will be provided to the participants at the end of each study visit to compensate the transportation cost for the scheduled visits.

Study Visit	Amount of Travel Allowance (RM)
1	100
2	100
3	100
4	150

Discontinuation from the study intervention does not mean discontinuation from the study and remaining study procedures should be completed as indicated by the study protocol. If a clinically significant finding is identified (including, but not limited to changes from baseline) after enrolment, the investigator or qualified designee will determine if any change in participant management is needed. Any new clinically relevant finding will be reported as an adverse event (AE).

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Participant unable to receive multivitamin supplements or placebo for 10 days per 24 weeks.

The reason for participant discontinuation or withdrawal from the study will be recorded. Participant who sign the informed consent form and are randomized but do not receive the active intervention or placebo may be replaced. Participant who sign the informed consent form and are randomized and receive the active intervention or placebo and subsequently withdraw, or are withdrawn or discontinued from the study will not be replaced.

A participant is considered as lost to follow-up if he fails to return for week 24 (post-intervention visit) and week 32 (follow-up visit) scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fail to return to the study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 1 week and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or study site staff will make every effort to regain contact with the participant (where possible, 2 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or email). These contact attempts should be documented in the participant's study file.
- Should the participant continue to be unreachable, he will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

18. Data Management

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harminosation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

19. Statistical Methods

20.1 Outcome

The primary and secondary outcome endpoints at baseline, Day 42, Day 84, Day 168 will be analysed using a two way repeated measures ANOVA for treatment and time. The primary and secondary outcome endpoints will also be assessed for statistical difference within-groups (change from

baseline) and between groups by t-tests. The correlations will be calculated using the Pearson Correlation Co-efficient. Effect sizes are reported as Eta squared (η^2).

20.2 Additional Analyses

Not applicable.

20.3 Analysis Population and Missing Data

The data obtained will be analysed using the intention-to-treat approach for both active intervention and placebo groups, considering all participants as randomized regardless of whether they received the randomized treatment or placebo. To prevent attrition bias, outcome data obtained from all participants are included in the data analysis, regardless of protocol adherence.

METHODS: MONITORING

20. Data Monitoring

21.1 Formal Committee

Data monitoring committee (DMC) has not been set up for this clinical trials as the intention of the study the effect of high-dose-vitamin-B multivitamin supplement on neural connectivity and oxidative metabolism in healthy adults. In addition, the ingredients presence in the active intervention has been studied in previous studies and has been shown not to elevate safety risk of the study population.

21.2 Interim Analysis

No interim analysis will be performed as this is a short-duration (24 weeks) trial study. The data analysis will be performed upon completion of the study.

21. Harms

22.1 Adverse Event (AE)

In our study, an adverse event (AE) will be defined as any untoward medical occurrence in a participant without regard to the possibility of a causal relationship. An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Adverse events will be collected after the participant has provided consent and enrolled in the study. If a participant experiences an adverse event after the informed consent document is signed (entry) but has not started to receive study intervention, the event will be reported as not related to study intervention. All adverse events occurring after entry into the study will be recorded. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study. An adverse event that meets the criteria for a serious adverse event (SAE) during study enrolment will be reported to MREC as an SAE.

22.2 Serious Adverse Event (SAE)

Serious adverse event for this study is any untoward medical occurrence that is believed by the investigators to be causally related to study-drug and results in any of the following: Life-threatening

condition (that is, immediate risk of death); severe or permanent disability, prolonged hospitalization or any other significant hazard. Serious adverse events occurring after a participant is discontinued from the study will NOT be reported unless the investigators feels that the event may have been caused by the study intervention or a protocol procedure. Investigators will determine relatedness of an event to study intervention based on a temporal relationship to the study intervention as well as whether the event is unexpected or unexplained given the participant's clinical course, previous medical conditions and concomitant medications.

22.3 Pre-existing Condition

A pre-existing condition is one that is present at the start of the study. A pre-existing condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

22.4 General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a pre-existing condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

22.5 Post-study Adverse Event

All unresolved adverse events should be followed by the Investigator until the events are resolved, the participant is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the Investigator should instruct each participant to report any subsequent event(s) that the participant, or the participant's personal physician, believes might reasonably be related to participation in this study. The Investigator should notify the Sponsor of any death or adverse event occurring at any time after a participant has discontinued or terminated study participation that may reasonably be related to this study. The Sponsor should also be notified if the Investigator should become aware of the development of any diseases and unwanted conditions after participation in the study.

22.6 Abnormal Laboratory Values

A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

22.7 Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event. Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a pre-existing condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical Investigator.

22.8 Adverse Events Classifications

The relationship of the adverse event to the study intervention will be described as one of the following: (3 tier system)

- Probable - Good reasons and sufficient documentation to assume a causal relationship
- Possible - A causal relationship is conceivable and cannot be dismissed
- Unlikely - The event is most likely related to an etiology other than the study intervention

Severity of the Events will be defined as follow:

- Mild: Symptom(s) barely noticeable to subject or does not make participant uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief or symptom(s) but may be given because of personality of participant.
- Moderate: Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; participant is able to continue in study; treatment for symptom(s) may be needed.
- Severe: Symptom(s) cause severe discomfort; symptoms cause incapacitation or significant impact on participant's daily life; severity may cause cessation of study intervention; treatment for symptom(s) may be given and/or participant hospitalization.

The adverse events will be classified as described above by the PI and appropriately summarized.

22.9 Expected Adverse Events

There were no adverse events recorded during the previous clinical studies. The product has been well tolerated, however, some participants in the active treatment group might experience a slight stomach discomfort when taking the herbal supplement tablets in the absence of food.

22.10 Recording of Adverse Events

At each contact with the participant, the Investigator will seek information on adverse events by specific questioning and as appropriate, by examination. Information on all adverse events will be recorded immediately in the appropriate adverse event module of the CRF. All clearly related signs, symptoms, and abnormal diagnostic procedures results if recorded in the CRF, will be grouped under one diagnosis. All adverse events occurring during the study period will be recorded. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the reasonable final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study intervention or study participation will be recorded and reported immediately.

22.11 Reporting of Adverse Events

A serious adverse event must be reported by telephone within 24 hrs of becoming aware of the event to the sponsor. A Serious Adverse Event (SAE) form (initial report) must be completed by the Investigator and faxed/emailed to the sponsor within 48 hours. The Investigator will keep a copy of the SAE form in file at the study site. All serious adverse events (including follow-up information) must be submitted to the MREC within 7 working days. Copies of each report and documentation of MREC notification and receipt will be kept in the PI's binder.

22. Auditing

Auditing from the IRB and authority agency will be performed independent of principal investigator, research team and sponsor.

ETHICS AND DISSEMINATION

23. Research Ethics Approval

This protocol and the template informed consent forms contained in Appendices will be reviewed and approved by the sponsor and IRB with respect to scientific content and compliance with applicable research and human subject's regulations. The protocol, site-specific informed consent forms (local language and English versions), participant education and recruitment materials and other requested documents will also be reviewed and approved by the IRB.

24. Protocol Amendments

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the participant or may affect participant safety, including changes of study objectives, study design, participant population, sample sizes, study procedures, or significant administrative aspects will require a formal amendment to the protocol. Such amendment will be agreed upon by principal investigator, sponsor and approved by the IRB prior to implementation. Administrative changes of the protocol are minor corrections and/or clarifications that have no effect on the way the study is to be conducted. These administrative changes will be agreed upon by principal investigator and sponsor and will be documented in a memorandum. The IRB may be notified of administrative changes at the discretion of principal investigator.

25. Consent

Consent forms will be IRB approved. Principal investigator or research staff will introduce the trial to participants regarding the main aspects of the trial and answer any questions that may arise. Participants will also receive information sheets. A verbal explanation will be provided in terms suited to the participant's comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their family or surrogates or think about it prior to agreeing to participate. The participants will sign the informed consent document prior to any procedures being done specifically for the study. Participants will be informed that participation is voluntary and that they may withdraw from the study at any time, without prejudice. A copy of the informed consent document will be given to the participants for their records. The informed consent process will be

conducted and documented in the source document (including the date) and the form signed, before the participants undergoes any study-specific procedures. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. The following consent materials are submitted with this protocol:

- Subject Information Sheet
- Informed Consent Form

26. Confidentiality

Participant confidentiality and privacy is strictly held in trust by the investigator, their staff(s) and the sponsor(s). This confidentiality is extended to cover testing of biological samples and MRI scanning in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor and participant. All research activities will be conducted in private setting as much as possible. In order to maintain subject confidentiality, only the site number, subject number and subject initials will be identified in the CRFs and other documentation submitted to the Sponsor. The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or pharmaceutical company supplying study products may inspect all documents and records required to be maintained by the investigators, including but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records. The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

27. Premature Termination or Suspension of Study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to investigators, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements; or Data that are not sufficiently complete and/or evaluable
- Determination of futility

Study may resume once the concerns about safety, protocol compliance and/or data quality are addressed and satisfy the sponsor and/or IRB.

28. Declaration of Interests

Principal investigator and research team declare no conflict of interest for the overall trial and each study site.

29. Access to Data

Principal investigator and research team will be given access to the cleaned data sets.

30. Ancillary and Post-Trail Care

The Taylor's University and sponsor will have insurance to cover for non-negligent harm associated with the protocol. This will include cover for additional health care, compensation or damages whether awarded voluntarily by the Sponsor or by claims pursued through the courts. Incidences judged to arise from negligence (including those due to major protocol violations) will not be covered by study insurance policies.

31. Dissemination Policy

31.1 Trial Results

Every attempt will be made to reduce to the interval between the completion of data collection and the release of the study results. We expect to take about 2 to 3 months to compile the final results and paper for an appropriate journal. The study results will be released to the participating physicians, referring physicians, participants and the general medical community

31.2 Authorship

Only person that has provided substantive contributions to the design, conduct, interpretation, and reporting of a clinical trial are recognised through the granting of authorship on the final trial report.

31.3 Reproducible Research

The full protocol, participant-level dataset and statistical code will not be make public available.

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