A study protocol to assess the effectiveness of tocotrienol-rich fraction in older adults : a randomised, double-blind, placebo-controlled

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Abstract

Background: Tocotrienol, a naturally occurring form of vitamin E, has been extensively studied for its potent antioxidant, anti-inflammatory, and immune-stimulating properties. However, the clinical impact of tocotrienol supplementation on older adults' overall health and well-being remains relatively unexplored.

Objective: This research aims to investigate the efficacy of tocotrienol-rich fraction (TRF), on various health parameters associated with general well-being in individuals aged between 50-75 years

Methods: The present study is a randomised, double-blind, placebo-controlled trial designed to investigate the effectiveness of TRF supplementation on overall health in healthy elderly individuals. The study aims to assess the impact of a daily dosage of 200mg of TRF over a period of 6 months. A total of 220 participants are enrolled in the study, with half receiving the placebo and the other half receiving TRF supplementation. The study comprises three endpoints: baseline, 3 months, and 6 months. At each endpoint, various measurements are taken to evaluate different aspects of health. These measurements include blood biochemistry assessments such as liver function tests, renal profile, lipid profile, and full blood count. Oxidative stress markers, including malondialdehyde, advanced glycation end-products, protein carbonyl, and isoprostane, are also evaluated. Immune response markers such as interleukin-6 and tumour necrosis factor-alpha are assessed. Satiety regulation is examined through measurements of leptin and ghrelin. Body composition and skin health parameters, including wrinkling, pigmentation, elasticity, hydration, and sebum secretion, are evaluated. Additionally, arterial stiffness is assessed by arteriography, bone mineral density is measured using dual x-ray absorptiometry, and cognitive function is assessed through the Montreal Cognitive Assessment, Rey Auditory Verbal Learning Test, and digital span, are measured at baseline and at the 6-month endpoint.

Results: NIL.

Conclusions: By comprehensively evaluating these health aspects, this study seeks to provide valuable insights into the potential benefits of tocotrienol supplementation for promoting the overall health and well-being of the ageing population. Clinical Trial: National Medical Research Register (NMRR), no. NMRR19-2972-51179

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A study protocol to assess the effectiveness of tocotrienol-rich fraction in older adults : a randomised, double-blind, placebo-controlled

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Abstract

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Discussion: By comprehensively evaluating these health aspects, this study seeks to provide valuable insights into the potential benefits of tocotrienol supplementation for promoting the overall health and well-being of the ageing population.

Trial registration: National Medical Research Register (NMRR), no. NMRR19-2972-51179

Keywords:
tocotrienol, skin, cognition, brain, weight, satiety ageing, oxidative stress, immune, inflammation

Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items.

Title {1}	A study protocol to assess the effectiveness of tocotrienol-rich fraction in elderly: a randomised, double-blinded, placebo-controlled
Trial registration {2a and 2b}	NMRR19-2972-51179
Protocol version {3}	Protocol version 3.0 (01/16/2020)
Funding {4}	This research was funded by KL-Kepong Oleomas Sdn. Bhd. (FF-2020-068) and Universiti Kebangsaan Malaysia's matching grant (DPK-2020-008).
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Name and contact information for the trial sponsor {5b}	KL-Kepong Oleomas Sdn. Bhd., Level 8, Menara KLK, No. 1, Jalan PJU 7/6, Mutiara Damansara, Petaling Jaya, 47810, Selangor, Malaysia
Role of sponsor {5c}	The funding resource (KL-Kepong Oleomas Sdn. Bhd.) has no role in the collection, analysis, and interpretation of data manuscript.

1. BACKGROUND

Ageing is a progressive process characterized by the gradual loss of tissue and organ function over time [1]. Two theories of ageing include the free radical theory of ageing, which later evolved into the oxidative stress theory of ageing, and the theory based on low-grade inflammation (inflammageing) in older adults. The oxidative stress theory is based on the hypothesis that age-related functional declines occur as a result of the accumulation of oxidative damage to macromolecules (such as lipids, DNA, and proteins) caused by reactive oxygen and nitrogen species (RONS) [2]. Additionally, chronic low-grade inflammation has been associated with the development of age-related chronic diseases [3].

Cumulative scientific evidence has convincingly shown that tocotrienol , another member of vitamin E, possesses pronounced antioxidant and anti-inflammatory functions [4-7]. These properties make tocotrienol a potential candidate for slowing down the ageing process and extending lifespan. Tocotrienol is a naturally occurring vitamin E and is primarily found in palm oil [8]. They can be further distinguished into four isomeric forms: alpha, beta, gamma, and delta, depending on the location and number of methyl groups on the chromanol ring. Tocotrienol has been reported to ameliorate a number of inflammatory markers, including Interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α) and C-reactive protein (CRP) [9-11]. These markers are consistently associated with age-related chronic diseases and disability [3]. IL-6 is a cytokine produced by various cells, including immune cells, vascular endothelial cells, adipocytes and skeletal muscle, and has both anti-inflammatory and pro-inflammatory properties. TNF-α, another cytokine, is mainly produced by macrophages and other cell types, While CRP is an acute-phase protein produced by the liver in response to elevated IL-6 levels [12-14].

In older adults, there is often an imbalance between excessive free radicals with low antioxidant levels [15]. With advancing age, the accumulation of RONS leads to post-transcriptional modifications and oxidative damage, such as advanced glycation end products (AGEs), malondialdehyde (MDA), protein carbonyl (PC), F2-isoprostanes (F2-IsoPs) [16]. High levels of oxidative damage and inflammatory cytokines are associated with low cognitive performance, osteoporosis, atherosclerotic characteristics, skin disorders, and renal problems in elderly subjects [17-19]. A previous study reported that tocotrienol-rich fractions (TRF) can improve antioxidant enzyme activities and glutathione levels in females aged between 50-55 years [20]. In the same

study, TRF supplementation was found to reduce MDA levels as early as 3 months while DNA damage was reduced in females after 6 months of supplementation [21].

A growing body of preclinical evidence strongly supports the wide range of health benefits that tocotrienols offer to humans. TRF supplements with a complete spectrum of tocotrienol isomers has been found to alleviate symptoms of allergic rhinitis [22], enhance mental health and cognition [23], reduce inflammation and oxidative stress in ulcerative colitis [24], and mitigate ultraviolet-induced skin inflammation [25]. Furthermore, related isomers derived from the same source have demonstrated benefits in cardiovascular, liver, and metabolic functions [26,27], while exhibiting inhibitory effects on the growth of gastric cancer [28], prostate cancer [29], and breast cancer [30]. The findings from these studies provide a compelling basis to initiate a clinical trial aimed at evaluating the impact of TRF on the well-being of healthy individuals. The objective of this randomised double-blinded, placebo-controlled study is to evaluate the efficacy of TRF supplement on health indices of older adults who have been reported to be at risk of reduced antioxidant levels and low-grade chronic inflammation, factors that contribute to an increased susceptibility to chronic diseases [31]. This study design offers the advantage of establishing causality by assessing the efficacy of TRF compared to a placebo in older adults. The outcomes of this trial will provide crucial insights into the potential benefits of TRF supplements in enhancing the health and well-being of the elderly population.

Preclinical evidence strongly supports the role of tocotrienols as antioxidants that effectively neutralize free radicals, including reactive oxygen and nitrogen species, thereby reducing oxidative stress [8, 32-37]. Moreover, various scientific studies have highlighted the capacity of tocotrienols to mitigate inflammation by targeting crucial transcription factors such as NF-κB, the Stat3 pathway, and other cytokine regulators integral to the inflammatory signalling cascade [4, 24, 37, 38-40]. Furthermore, these scientific investigations have illuminated tocotrienols' ability to enhance skin health through UV protection and skin-lightening effects, both in preclinical and clinical models [25, 41-44]. Tocotrienols have also demonstrated their potential in improving lipid profiles among individuals with dyslipidaemia and hypertension [39, 45-50]. Additionally, research papers have revealed the regulation of vitamin E in leptin expression [51-54]. The wealth of scientific evidence supports tocotrienols in promoting osteoblast survival and proliferation through free radical protection. On the other hand, they inhibit osteoclasts by downregulating the mevalonate pathway. Moreover, tocotrienols have exhibited their capacity to modulate the expression of genes favouring bone formation [55-64]. Furthermore, scientific findings indicate that tocotrienols can modulate gene expression in the brain, potentially enhancing memory and motor functions while delaying the progression of Alzheimer's disease. Notably, alpha-tocotrienol at nanomolar concentrations has been demonstrated to attenuate both enzymatic and nonenzymatic mediators of arachidonic acid metabolism and neurodegeneration [65-67].

To establish an appropriate dosage and duration for the clinical trial, a systematic search of human studies involving vitamin E supplementation in healthy subjects, spanning the years 1997 to 2019 was conducted. A comprehensive search, using keywords such as 'human,' 'vitamin E,' 'tocopherol,' and 'tocotrienol' on PubMed database, yielded nine published papers. These studies shed light on the dosages and durations of tocotrienols that is suitable for the current clinical trial. In a comparative study investigating the impact of supplementation with TRF and α-tocopherol (αTP) on gene expression in healthy older adults, the administration of αTP (400 IU/day) and TRF (150 mg/day) over a period of 6 months was found to influence pathways related to immune response, drug response, cell adhesion, and signal transduction. Although other pathways were also modulated, the effects were more pronounced in certain areas [68]. Notably, the study revealed that TRF and α-TF supplementation demonstrated similar antioxidant effects in older adults, with TRF showing more significant impacts in females [69]. Moreover, supplementation with TRF (150 mg/day) for 6 months led to alterations in plasma levels of apolipoprotein A-I precursor, apolipoprotein E precursor, and C-reactive protein precursor in both young and older individuals [70].

Another investigation focused on the effects of supplementing with tocotrienol-rich fraction at a dosage of 400 mg/day for 2 months on the immune response to tetanus toxoid immunization in normal healthy volunteers. The findings indicated that TRF exhibited immunostimulatory effects [71]. In a randomized controlled trial spanning 6 months, daily supplementation of 150 mg/day TRF was associated with improvements in lipid profile and oxidative status in healthy older adults [72]. Interestingly, a separate trial found that daily supplementation with tocotrienol-rich fraction (150 mg/day) for 6 months did not induce immunomodulatory changes in healthy human volunteers [73]. A much earlier clinical trial, comparing 50- and 100 mg vitamin E supplementation over 6 months, suggested that the higher dose (100 mg) had a more pronounced effect on cellular immune function in noninstitutionalized elderly subjects [74]. Moreover, a 4-month supplementation regimen of 60-800 IU vitamin E daily had no adverse effects on various health parameters, including general health, nutrient status, liver enzyme function, thyroid hormone concentrations, creatinine concentrations, serum autoantibodies, killing of Candida albicans by neutrophils, and bleeding time in healthy subjects aged above 65 years [75]. In another randomized controlled trial, elderly subjects consuming 200 mg/d of vitamin E for 4 months exhibited an increase in delayed-type hypersensitivity skin response and an elevation in antibody titer to hepatitis B [76].

Following the review of the literature, a supplementation regimen of 200 mg per day for 6 months was selected. While no published human studies have reported any serious adverse effects attributed to tocotrienols, it's worth noting that in animal studies, tocotrienol doses of 500 and 1,000 mg/kg body weight (administered orally) were found to increase bleeding and clotting times in mice during sub-acute (14 days) and sub-chronic (42 days) investigations [77]. When these doses are converted to Human Equivalent Dosage (HED), they equate to 2,400 and 4,800 mg in humans. This discovery implies that high doses of tocotrienols (>2,400 mg) should be used cautiously, particularly by individuals with a tendency to bleed or those taking anticoagulants. Consequently, individuals who are currently on anticoagulant or anti-thrombotic medication are ineligible for participation in this research.

While several human studies have explored the potential benefits of tocotrienols, there is a noticeable dearth of published, comprehensive data that substantiates their advantages specifically in older adults. To address this knowledge gap, a randomised, double-blind, placebo-controlled study, a widely recognized 'gold standard' in intervention research is designed [78]. This research design excels in establishing causal relationships, enabling us to demonstrate the efficacy of tocotrienols in older adults compared to a placebo. The connection between oxidative stress, chronic low-grade inflammation, and the ageing process, as well as age-related diseases, has been firmly established through extensive epidemiological studies involving older adults [79]. Consequently, several key investigative areas, including antioxidant and inflammation levels, arterial stiffness, appetite regulation, skin parameters, cognitive functions, and bone mineral density, as focal points were chosen for this research.

Aim of this study

The comprehensive objectives of this research involve the evaluation of the effectiveness of TRF in enhancing antioxidant levels and reducing inflammation among individuals aged 50 years and older. Furthermore, changes in appetite hormones, arterial stiffness, and skin parameters over a 3- and 6-month supplementation period are monitored relative to baseline measurements. In addition, alterations in bone mineral density following 6 months of tocotrienol supplementation are determined, and potential enhancements in cognitive function between baseline and the 6-month point are investigated. Finally, the influence of supplementation on vitamin E levels within the body is assessed after 3 and 6 months of TRF supplementation.

Methods

Study setting

Healthy men and women of the age between 50 and 75 from the Klang Valley which is cantered in the federal territories of Kuala Lumpur and Putrajaya in Malaysia are recruited and divided into two cohorts which are supplemented with either placebo or tocotrienols for 6 months.

Eligibility criteria

Participants in this research must meet specific health criteria, including being generally healthy as determined by physical examination and blood lab tests, with satisfactory liver and renal function tests. Eligible participants can be of either gender, aged between 50 and 75 years. They should not have allergies to palm oil and vitamin E and should not have consumed vitamin E supplements in the past 3 months. Furthermore, they must be willing and able to adhere to the study's visit schedule and procedures, reside within a geographically feasible proximity for adequate follow-up (as determined by the investigator), and have a clear understanding of the study protocol, as indicated by their signed informed consent forms.

Participation in this research is restricted based on several exclusion criteria. Individuals with fat malabsorption, as well as those managing chronic conditions such as cardiac diseases, neurological diseases, diabetes, HIV infection, or psychiatric illness/social situations, are not eligible. Moreover, individuals adhering to a vegan diet, those who have smoked within the past 3 months, individuals awaiting surgery or who have undergone surgery within the same timeframe, or those with a current or past history of drug, alcohol abuse, or cancer, do not meet the study's eligibility requirements. Pregnant and lactating women, individuals with a history of bleeding tendencies or conditions that predispose to bleeding (e.g., thrombocytopenia, abnormal liver function, liver diseases such as chronic hepatitis, or gastrointestinal ulcers), those taking antibiotics or other medications, or dietary supplements that may interfere with tocotrienol action, individuals with a predisposition to inherited blood/circulation disorders, and those using anticoagulants and antithrombotic drugs are also ineligible for participation in this research.

Interventions

This research encompasses the evaluation of two investigation products that are supplied by KL-Kepong Oleomas Sdn. Bhd. The first product, known as DavosLifeE3 Complete (50mg), contains 50mg of tocotrienols that are sourced from palm oil and meet the specifications outlined in the US FDA GRAS exemption claim 21 CFR 170.36 (c) [80]. The second product, Placebo Softgel, serves as a vital control within the study.

Outcomes

The study encompasses a multifaceted array of assessments and procedures (Figure 1). Firstly, blood samples (15 ml) are collected as baseline samples for blood biochemistry analyses. Tubes containing samples are labelled with the subject's code, trial ID, and date for accurate identification. Of the collected blood samples, 4 ml is routed to the chemical pathology laboratory for further analysis. The remaining samples are stored appropriately on ice or in a 4^{o}C refrigerator until they undergo centrifugation, facilitating the isolation of plasma samples. These collected plasma samples are meticulously apportioned into 1ml storage tubes and immediately stored in a -80 ^{o}C freezer. This storage method ensures that the samples are preserved for the analysis of oxidative stress markers (MDA, AGE, F2-IsoPs, PC), inflammatory markers (TNF-α, IL6), vitamin E, leptin, and ghrelin levels, all to be conducted within 12 months of storage (Table 1).

Furthermore, the assessment of arterial stiffness is conducted by skilled laboratory assistants through arteriography during the subjects' visits at both the 0 and 6-month time points. Total body fat and lean mass measurements are accomplished using an Inbody (USA) device during the subjects' visits at 0, 3, and 6 months.

Subsequently, subjects undergo a cognitive function assessment, which includes the Montreal

Cognitive Assessment (MoCA), Rey Auditory Verbal Learning Test (RAVLT), and digit span. This assessment is performed with the assistance of a trained research assistant during the subjects' visits at 0 and 6 months.

In addition, the analysis of skin health is performed using Cutometer (Germany) and Visioscan (Germany) during subjects' visits at 0, 3, and 6 months. Finally, the measurement of bone mineral density is conducted using a dual-energy X-ray absorptiometry device (DEXA) at 0 and 6 months. To monitor changes in dietary habits, subjects complete Food Frequency Questionnaires (FFQ) during their visits at 0, 3, and 6 months.

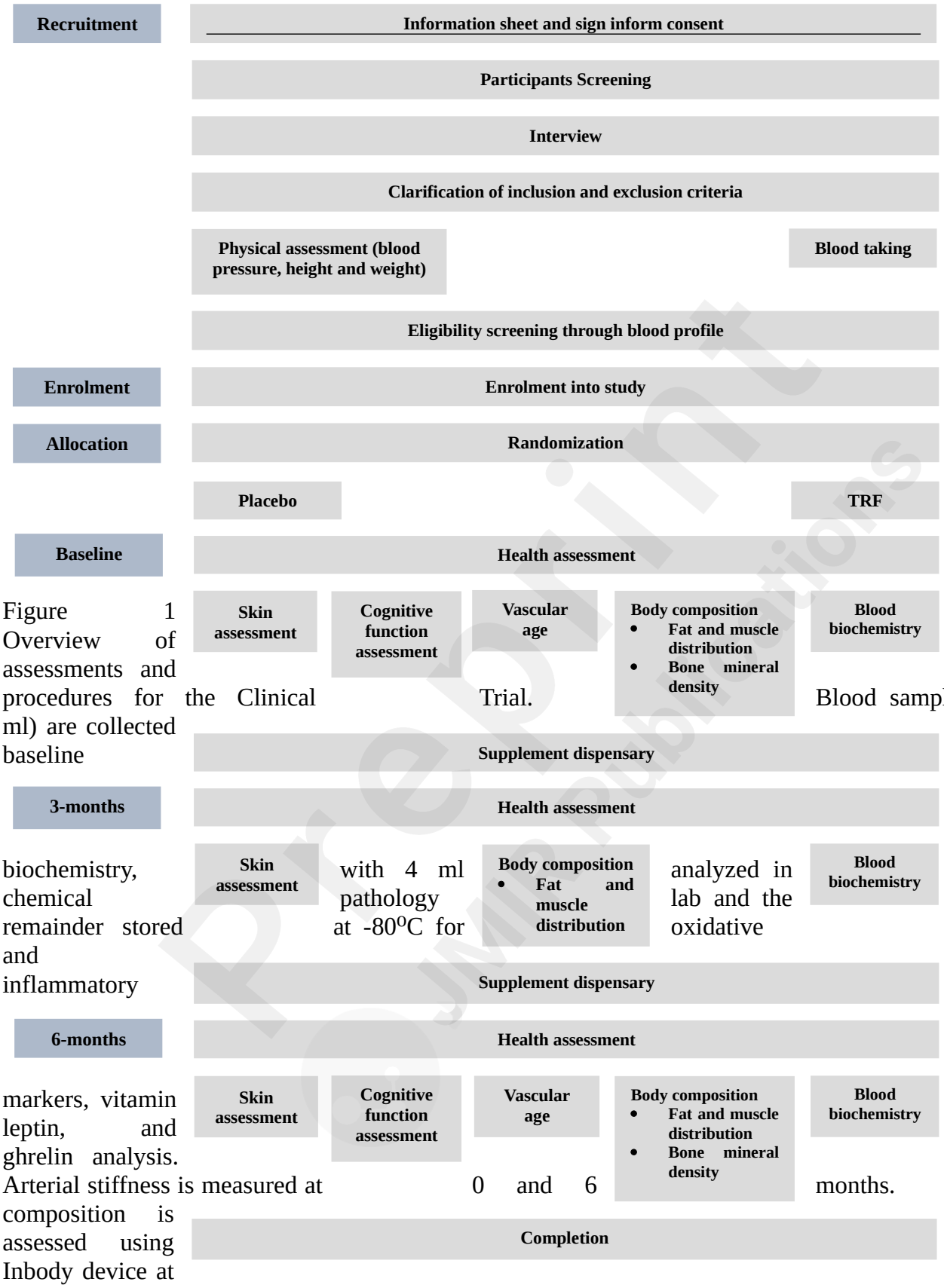

Figure 1 Overview of assessments and procedures for the Clinical Trial. Blood samples (15 ml) are collected for baseline biochemistry, chemical pathology with 4 ml remainder stored at -80^{o}C for and analyzed in lab and the oxidative stress and inflammatory markers, vitamin E, leptin, and ghrelin analysis. Arterial stiffness is measured at 0 and 6 months. Body composition is assessed using an Inbody device at 0, 3, and 6 months. Cognitive function tests (MoCA, RAVLT, digit span) are conducted at 0 and 6 months. Skin health is evaluated with Cutometer and Visioscan at 0, 3, and 6 months. Bone mineral density is measured with DEXA at 0 and 6 months. Dietary habits are monitored via Food Frequency Questionnaires at 0, 3, and 6 months.

Table 1 Summary of tests performed at 0, 3 and 6 months

No	Time points	Parameters	Sample	Methodology
1	Screening	**Blood biochemistry test** • Liver function (ALT, ALP, albumin, protein, bilirubin) • Lipid profile (TG, HDL, LDL, cholesterol) • Renal function (Na, K, Cl, Urea, Creatinine) • Full blood count (WBC, RBC, haematocrit, MCV, platelet) • Fasting glucose • C-reactive protein	Blood	Clinical chemistry analyser
		Physical examination (blood pressure, heart rate, BMI)		Blood pressure monitor, weighing and height scale
		Past medical history (medication, supplements, history of illness) **Social history** (smoking, alcoholic consumption, exercise, diet pattern)		
2	0, 3 and 6 months	**Physical examination** (blood pressure, heart rate, BMI)		Blood pressure monitor, weighing and height scale
		Body composition measurement • Body mass index • Segmental and visceral fat • Basal metabolic rate	Whole body	Body composition analyser
		Blood biochemistry test	Blood	Clinical chemistry analyser

		Oxidative stress markers		
		• Advanced glycation end-products (AGEs)	Blood	ELISA kit
		• Protein carbonyl (PC)		ELISA kit
		• F2-isoprostanes (F2-IsoPs)		ELISA kit
		• Malondialdehyde (MDA)		High performance Liquid Chromatography (HPLC)
		Inflammation markers		
		• Tumor necrosis factor-α (TNFα)	Blood	ELISA kit
		• Interleukin-6 (IL-6)		ELISA kit
		Weight Management		
		• Ghrelin	Blood	ELISA kit
		• Leptin		ELISA kit
		Vitamin E level	Blood	HPLC
		Skin assessment		
		• Elasticity	Face and Arm	Cutometer
		• Hydration		Corneometer
		• Transepidermal water loss	Face and Arm	Tewameter
		• Pigmentation		Mexameter
		• Sebum secretion	Face and Arm	Sebumeter
		• Wrinkles and Roughness		Visioscan
			Face and Arm	
			Face and Arm	
			Face and Arm	
5	0 and 6 months	**Arterial stiffness assessment**	Whole body	Arteriograph device /Photoplethysmography
		Bone density assessment	Whole Body	Dual-energy x-ray absorptiometry
		Cognitive function assessment		Questionnaires

	• MoCA • RAVLT • Digit Span

Participant timeline
Over the study period, three assessments at 0, 3, and 6 months, are carried out across various domains of inquiry, encompassing oxidative stress, inflammation, skin attributes, arterial stiffness, cognitive function, weight management, bone mineral density, and vitamin E levels. To ensure comprehensive oversight of the assessments, a delegation of authority log is established and formally endorsed by the respective investigators, each of whom possesses expertise in their specific area of assessment.

Sample Size
Sample size is calculated using Power and Sample Size Program. The total number of subjects calculated per group of experimental design is 93 when dichotomous outcomes is presumed with Type I error probability, α of 0.05 and power of 0.8. To maintain the power of study, 20% dropout rate of subjects is factored in. Consequently, a total of 110 subjects will be enrolled to receive the placebo, while an additional 110 subjects will be recruited to receive TRF supplements.

Recruitment
The selection of potential trial subjects is conducted by the Principal Investigator (PI) based on the inclusion and exclusion criteria. Informed consents are obtained from subjects by contract research assistance. During enrolment, general information (name, gender, age, address and contact information) is obtained. To ensure security and confidentiality, each subject is assigned a subject-specific code. The code is used to label the subject's laboratory results. Each subject is reimbursed with standard transportation fees.
Subjects who fail to meet the inclusion and exclusion criteria are defined as screening failures. The principal investigator (PI) maintains a Screening Log which includes screen failures to ensure a systematic selection of subjects.
Subjects are free to withdraw from the study at any time for any reason. Subjects may also be withdrawn at any time at the discretion of the investigator. Possible reasons for withdrawal include sample events, abnormal laboratory values, protocol violation, subject requiring the use of unacceptable concomitant medication, subject not complying with protocol procedures, subject developing a condition during the study that violates the inclusion/exclusion criteria, lost to follow up, death or any other reason in the investigator's opinion that would impede the subject's participation in the study.

Assignment of interventions
Tocotrienols and placebo supplements are prepared and labelled with codes by the sponsor. Assignment of supplements to subjects is made by chance through the drawing of lots by contract research assistants. The treatment is double-blinded throughout the study period until all data has been collected and analysed after which the supplementation codes are revealed to the PI by the sponsor. However, in case of any serious adverse event reported during the study period, the unmasking of code is done immediately.
A set of numbers from 1 to 60 is randomised using the randomization application for every block of 60 subjects. The subjects are assigned to the randomised number according to numerical sequence 1 to 60 in the list of randomised number. All even numbers are treated with the same IP while odd numbers are treated with another IP. Thus, every block randomization has an equal number of subjects for each IP. The randomization is repeated until 220 subjects are recruited.
The unblinding in emergency situations is only permitted in case of a suspected, unexpected serious adverse reaction (SUSAR) or other important adverse event, when the knowledge of the supplemented product in question is required for therapeutic decisions for the management

of the subject.
In the event of an emergency, an emergency decoding option is accessible to both the Investigator and designated personnel at the Sponsor. The responsibility for breaking the blind for individual subjects in emergency situations lies with the Investigator. If the need arises to break the blind, the Investigator will communicate this to the Sponsor. Before the treatment code is broken, the Investigator responsible for unblinding must document the reason and date for unblinding in the CRF to ensure proper documentation of the unblinding event, including a clear explanation of the reason for unblinding.
In the event that the Sponsor requires unblinding of the treatment, the reason and date of unblinding are recorded in the CRF. In situations where it is necessary to unblind an individual subject's treatment for the purpose of expedited reporting to the authorities, only designated individuals within the Sponsor, who are responsible for reporting this information, will have access to the identity of the product. Every effort is made to ensure that all other members of the clinical trial remain blinded throughout the entire duration of the trial.

Data collection and management
During the screening, potential subjects are assessed for eligibility to enter the study according to the inclusion and exclusion criteria. A total of 10 ml blood samples is taken from each volunteer after an overnight fast. The screening tests consist of comprehensive evaluations, encompassing blood lipid profile, liver function test, renal profile, full blood count, and fasting blood sugar measurements. Additionally, the records of past supplementation and medical history are reviewed to obtain a holistic understanding of the individual's health status. The results of blood tests are revealed to volunteers upon request. Volunteers who meet the inclusion criteria are selected for baseline sampling typically within a timeframe of 1 to 2 months following the screening process.
Eligible subjects are contacted to schedule appointments for data collection at baseline, 3rd and 6th month. During each visit, a comprehensive medical evaluation is conducted by a physician, encompassing medical history assessment, documentation of any concurrent medications, measurements of subjects' weight, height, resting blood pressure, pulse rate, and temperature, along with the evaluation of vital signs. Subsequently, blood samples are drawn from each subject for immediate analysis of blood biochemistry, and additional samples are preserved for future analysis of oxidative stress markers, inflammatory markers, vitamin E, leptin, and ghrelin levels. Furthermore, on the same day as the visit, assessments are performed for arterial stiffness, body fat composition, skin health, bone mineral density, and cognitive function.
The records of subjects and data of analyses are kept in separate folders according to the protocol and transferred into the CRF of each subject by the contract research assistants. The result for each assessment is monitored by specific investigators with expertise in the field, to facilitate comprehensive monitoring of the data.
A disclosure contractual agreement signed between the sponsors and investigators outline the sponsors' access rights to the trial dataset and source data. Both investigators and sponsors should collaborate and jointly author publications reporting on the trial's results to ensure an accurate representation of the results and the drawing of appropriate conclusions, thereby preserving the integrity of the findings. All data are reported in a collective manner with no reference to any subjects in the trial to ensure that the identity of subjects remain confidential.

Analyses
Statistical analysis will be conducted using SPSS software (Chicago, IL, USA). The Kolmogorov-Smirnov test will be performed to assess the normality distribution of the data. Type III sum of squares will be set to evaluate the hypotheses. A mixed-design ANOVA will be used to determine whether supplementation affects the blood biochemistry parameters

(inflammation and oxidative stress) and physical health (body composition, skin, cognitive function, and vascular health) at baseline, 3 months follow-up, and 6 months follow-up among the supplementation groups. Mauchly's test of sphericity will be conducted to assess homogeneity of variance and compare the means of the supplemented group to the placebo group. To control against an increase in the risk of Type I errors during significance evaluation, Tukey's post hoc test for multiple comparisons will be applied. Dunnett's pairwise multiple comparison test will be utilized to assess the changes of the means within groups. Prior to statistical analysis, any missing data will be excluded. All data will be presented as mean ± standard error of the mean, with statistical significance defined as $p < 0.05$.

Monitoring

Subjects are instructed to maintain their regular lifestyle throughout the study duration. It is recommended that they adhere to their usual routine regarding the number of meals and dietary plan. If there is a necessity to use any medication, they are required to inform the PI to ensure proper documentation in the CRF.

To ensure compliance, subjects are requested to submit the provided supplement bottles during their visits at the 3rd and 6th month. Additionally, plasma vitamin E levels are measured at 0, 3, and 6 months to assess the subjects' response to supplementation. In the event of suspected side effects or adverse reactions, subjects should promptly report to the PI and seek immediate medical assistance. The PI will assess whether the observed effects are related to the trial and determine if the subject should be excluded from the study accordingly.

Ethics and dissemination

This research upholds the principles of Good Clinical Practice (GCP) and adheres to the pertinent regulatory guidelines established by the Malaysia Ministry of Health (MOH). The research is approved by the Research Ethics Committee of Universiti Kebangsaan Malaysia (UKM), registered with the Malaysia National Medical Research Register (NMRR) and National Pharmaceutical Regulatory Agency (NPRA). Funding for the study is provided by KL-Kepong Oleomas Sdn. Bhd., with the research itself being independently conducted by scholars and experts from a higher learning institution. Before the clinical trial commenced, the two parties sign a distinct legal agreement designed to safeguard the rights of the sponsor and researchers involved.

It is important to note that the investigators have no financial or other competing interests in relation to the overall trial, ensuring impartiality and scientific integrity. Any alterations to the protocol are communicated by the investigators to both the Research Ethics Committee and the sponsor prior to implementation.

Upon completion of the trial, the PI and sponsor collaborate in publishing the results and deriving pertinent conclusions, all while ensuring the strict confidentiality of subjects' personal information.

The PI is responsible for retaining the data for a minimum duration of seven years from the date of closure of the database. The sponsor possesses exclusive rights to utilize the data for commercial purposes, except for any personal information related to the subjects. Concurrently, researchers are granted the privilege to employ the data for non-commercial purposes, encompassing but not limited to patient care and treatment, academic pursuits, and publication.

Discussion

The five years clinical trial was designed in accordance with the guidelines outlined by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) and registered in the National Medical Research Register (NMRR) of Malaysia. Prior to the initiation of the trial, the investigators and sponsor signed a mutual confidentiality agreement. This agreement serves to safeguard against any unauthorized use, disclosure, publication, or dissemination of confidential information by both parties involved. Separately, a comprehensive research agreement was signed, addressing the roles of the investigators and sponsor, ownership of liabilities, indemnification and insurance, quality assurance, record-keeping, reporting, access, intellectual property, and publication and authorship.

The trial commenced in 2019 after ethical approval was obtained. Notably, although sponsored by a commercial company, the trial is conducted entirely by the institution independently of the sponsors where subject recruitment, sampling and analyses are performed by the investigators who are blinded from subjects' identification to prevent bias. Volunteers undergo random screening and are recruited based on their fulfilment of the inclusion criteria. Similarly, the administration of investigational products is also randomised.

The trial protocol was collaboratively designed by investigators from diverse backgrounds, each bringing their expertise in the relevant measured parameters. Through careful consideration and discussion, the team determined the optimal parameters to be measured and the appropriate time points at which these measures were most likely to detect changes effectively. As a result, blood tests, skin assessment, and body composition were assessed at three-time points, while parameters such as bone density, arterial stiffness, and cognitive function were measured exclusively at the baseline (0 months) and endpoint (6 months) evaluations.

The MoCA was utilised under the licensing agreement with MoCA Clinic & Institute, ensuring that only trained research assistants administered the test to the subjects. The RAVLT is a widely recognised measure that assesses an individual's capacity to encode, integrate, retain, and retrieve verbal information across various stages of immediate memory [81]. As a result, this assessment tool is valuable for evaluating the impact of interference stimulus, delayed memory, and recognition. Additionally, the digit span test, which gauges the ability to maintain and manipulate a limited amount of information within a readily accessible state for short durations, typically less than 30 seconds, provides crucial insights when assessing working memory [82].

The dosage of TRF was determined based on previous reports that demonstrated its efficacy while not reporting any adverse events. Careful consideration was given to selecting an appropriate dose to ensure safety and prevent potential toxicity, as TRF is a fat-soluble compound, and excessive dosage can lead to adverse effects. Research in rats has shown that oral administration of vitamin E supplement was not safe for the liver and kidney [83]. Nevertheless, considering the ongoing changes in population lifestyles and dietary habits, it becomes imperative to conduct a comprehensive review of the TRF dosage for future clinical trials especially for therapeutic purposes.

On average, the screening process typically lasts around 2 hours. According to the latest data, approximately 60% of the volunteers successfully met the inclusion criteria and became eligible for recruitment into the study. The primary reasons for volunteer exclusions were elevated levels of total cholesterol, LDL, fasting blood glucose, and blood pressure. On average, each subject requires approximately 3 hours to complete all assessments, excluding blood tests. To accommodate the fasting requirement for blood withdrawal and arterial stiffness assessment, these tests are conducted first. Subsequently, the remaining assessments are performed simultaneously at different stations, allowing subjects to visit any available station. To minimise waiting time, a maximum of five subjects are scheduled for a sampling

day. This ensures that subjects do not experience prolonged waiting periods.

Throughout the treatment period, the research assistants monitor the compliance of the subjects through regular phone calls and short messages. However, it is important to note that the effectiveness of this monitoring method relies on the subjects' availability to respond to or read the messages. Non-compliance cases were primarily attributed to the subjects' difficulty in remembering to take the supplement, which is evident when they return the bottles during the 3rd- and 6th-month visits with a higher number of remaining soft gels than expected. The data from subjects who were non-compliant with the trial is still analysed. However, it is specifically noted in the CRF so that any potential outliers in data analysis can be identified later.

The findings of this study will further extend our current knowledge in many ways. As the elderly population continues to rise globally [84], understanding the efficacy of TRF supplementation in promoting healthy aging becomes increasingly imperative. The utilization of a randomized, double-blind, placebo-controlled study protocol to assess the effectiveness of TRF in the elderly offers numerous advantages that significantly enhance the credibility and reliability of the research findings. Firstly, the randomization process ensures an unbiased allocation of participants into treatment and control groups, minimizing the influence of confounding variables and increasing the validity of the study results. Secondly, the double-blinded nature of the trial eliminates both participant and researcher bias, as neither knows who is receiving the active intervention or the placebo, thereby preventing any preconceived notions from influencing outcomes. This rigorous blinding protocol ensures the integrity of the data collected and provides a more accurate assessment of the true effects of TRF supplementation. Finally, the inclusion of a placebo group enables researchers to distinguish between the specific effects of TRF and any placebo effects, ensuring that observed improvements in health outcomes can be confidently attributed to the intervention. Overall, by adhering to such a meticulous study protocol, researchers can generate robust evidence regarding the efficacy of TRF in promoting health and well-being among the elderly, ultimately informing clinical practice and guiding future research endeavours.

There are some possible limitations of the present study. One significant limitation is the potential for dropout or non-compliance among elderly participants, which can compromise the integrity of the study results and introduce bias. Elderly individuals may face unique challenges such as cognitive impairment, mobility issues, or comorbidities that affect their ability to adhere to the study protocol consistently. Moreover, the relatively long duration often required for such trials increases the likelihood of attrition over time. Additionally, the strict inclusion and exclusion criteria necessary to ensure the internal validity of the study may limit the generalizability of the findings to broader elderly populations with diverse characteristics and health profiles [85]. Thus, researchers must remain cognizant of these limitations and employ strategies to mitigate their impact on the study outcomes.

In conclusion, our study holds immense promise for advancing our understanding of TRF potential health benefits. While acknowledging the inherent challenges and limitations associated with conducting trials in elderly populations, the insights gleaned from this study have the potential to significantly impact clinical practice. Ultimately, by generating robust evidence regarding the efficacy of TRF supplementation in promoting healthy aging, this study protocol has the potential to contribute valuable insights to the field of geriatric medicine, paving the way for improved strategies to enhance the quality of life and well-being of elderly individuals worldwide.

List of abbreviations

AGE	advanced glycation end-products

CRF	case report form
CRP	C-reactive protein
DEXA	dual-energy X-ray absorptiometry
F2-IsoPs	F2-isoprostanes
IL6	Interleukin-6
MDA	malondialdehyde
MoCA	Montreal Cognitive Assessment
PC	protein carbonyl
PI	principal investigator
RAVLT	Rey Auditory Verbal Learning Test
TNF- α	tumour necrosis factor alpha
TRF	tocotrienol-rich fraction
αTP	alpha tocopherol

Declarations

Ethics approval and consent to participate

The Ethics Committee of UKM approved the clinical trial and the consented to participate before recruitment into the trial.

Consent for publication

The authors consent to the publication of this protocol.

Availability of data and materials

The data and materials are available upon request, subject to reasonable conditions.

Competing interest

J.A.G., W.Z.W.N, S.M., M.H.A.D., N.F.I., K.Y.C., A.A., M.H.M.Y. & M.M.M. declare that they have no competing interests.

J.R.E.N, M.H.Y.L. & W.N.Y, Y.W.U are from a commercial organization that sponsor the clinical trial.

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Authors' contributions

Conceptualization, J.A.G., W.Z.W.N, S.M., M.H.A.D., N.F.I., K.Y.C., A.A., M.H.M.Y., M.M.M., H.Y.L. & Y.W.N. Y.W.U.

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Formal analysis, J.A.G., N.F.I.

Investigation, N.F.I., F.A.S., N.M.M., A.A., M.H.M.Y., M.M.M.

Original draft preparation, J.A.G.

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APPENDICES

INFORMATION SHEET

Research Title:

A randomised, double-blinded, placebo-controlled study to evaluate the efficacy of oral supplementation of Tocotrienol-rich fraction (DavosLifeE3 Complete)

Introduction:

You are invited to participate in a research study. Before participating in this study, it is important that you take time to read and understand the information in this Information Sheet.

Purpose of Study:

Tocotrienol is a type of vitamin E compound. This vitamin is present abundantly in palm oil. DavosLifeE3 Complete is a patented tocotrienol enriched softgel supplement produced by KL-Kepong Oleomas Sdn. Bhd. Cumulative scientific evidences have convincingly shown that tocotrienols possess pronounced antioxidant and anti-inflammatory functions which may be beneficial for the slowing of ageing process. The purpose of the study is to investigate the general well-being effects of this vitamin E in the older adults since they are more susceptible to chronic diseases.

What will the study involve?
You will be requested to take 2 tablets of the given softgel twice daily; after lunch and dinner for 6 months. A total of 15 ml blood samples will be taken from you before the supplementation period begins and after 3 and 6 months for blood biochemistry tests. Apart from that, your bone mineral density and skin health will be assessed using approved clinical equipment before and after 6 months of supplementation. You will also be required to complete questionnaires to assess your cognitive function. Results of tests will be made known to you upon request. Please be advised to seek further investigation and management if there appears to be any abnormality in the test results.

Benefits:
There is unlikely to be a direct benefit for you in participating in this study. The supplements given may or may not help to improve your current health status. However, your participation could help us improve on anti-ageing interventions in the future.

Risks
The supplements given are in doses according to previous studies which have found benefits of tocotrienol to human health. They are unlikely to cause any side-effects.

Do you have to take part?
Participation in this study is voluntary. If you agree to take part, then you will be asked to sign the "Informed Consent Form". You will be given a copy of the form and this Information Sheet. Should you decide to participate, you can still withdraw from the study without penalty. Your data will not be used and will be discarded. The researcher may also remove you from the study for a specified reason.

Data & Confidentiality:
The data from this study will be made into a report which may be published. Access to the data is only by the research team and the REC UKM. The data will be reported in a collective manner with no reference to an individual. Hence your identity will be kept confidential.

Payment and compensation:
You do not have to pay to participate in this study. You will be given RM40 as travel reimbursement to the research site at each follow-up.

Who can I ask about the study?
If you have any questions, you can direct them to the research team. You can also contact the REC UKM for clarifications.
Name of PI
Phone Number of PI

INFORMED CONSENT FORM

Research Title: A randomised, double-blinded, placebo-controlled study to evaluate the efficacy of oral supplementation of Tocotrienol-rich fraction (DavosLifeE3 Complete)

Name of researchers

I, ……, IC No : ……,

- have read the information in the Patient Information Sheet including information regarding the risk in this study
- have been given time to think about it and all of my questions have been answered to my satisfaction.
- understand that I may freely choose to withdraw from this study at anytime without

reason and without repercussion
- understand that my anonymity will be ensured in the write-up.

I voluntarily agree to be part of this research study, to follow the study procedures, and to provide necessary information to the doctor, nurses, or other staff members, as requested.

…… ……
(Signature) (Date)

…… Witness (if any) …… (Signature) …… (IC Number) …… (Date)	…… Researcher …… (Signature) …… (IC Number) …… (Date)