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Effect of beetroot or beetroot plus vitamin C supplementation on cardiovascular function in patients with coronary artery disease: protocol for a double-blind, placebo-controlled, randomised trial

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ABSTRACT

Introduction Coronary artery disease (CAD), classified into the atherosclerosis category, is a prevalent cardiovascular disease worldwide that is associated with serious comorbidities and death. The purpose of this study was to evaluate the effect of beetroot/beetroot plus vitamin C on cardiovascular health status and function in patients with CAD.

Method and analysis A randomised, placebo-controlled, double-blind clinical trial to recruit 90 patients with CAD at the cardiac outpatient clinic and Imam Reza Hospital, Mashhad, Iran. Participants will be divided into three groups: (1) Those who receive 500 mg three times a day of beetroot capsules, (2) Those who receive 500 mg three times a day of beetroot plus vitamin C capsules, and (3) Those who receive placebo capsules three times a day for 4 weeks. Pulse wave velocity, Augmentation Index, heart rate, volume of oxygen (VO_2) max/VO_2 peak, peak heart rate, blood pressure, interleukin 6 (IL-6), high sensitivity C reactive protein, intercellular adhesion molecule, vascular cell adhesion molecule, lipid profile and anthropometry will be measured at the beginning and end of the intervention.

Ethics and dissemination This study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.MEDICAL.REC.1399.717). All participants will be asked to complete the consent form at the beginning of the study. The results will be actively disseminated through peer-reviewed journals and conference presentations.

Trial registration number Iranian Registry of Clinical Trials, IRCT20210217050393N1 (registered 16 May 2021).

INTRODUCTION

Cardiovascular disease has been recognised as a major cause of death in the last decade and is increasing every year.[1] Global deaths from cardiovascular diseases have increased dramatically since 1990.[2] Among cardiovascular diseases, one of the most prevalent types is coronary artery disease (CAD),[2 3]

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is a double-blinded, randomised, placebo-controlled study.
⇒ This study will compare the effect of beetroot capsules alone or in combination with vitamin C.
⇒ The follow-up period is not sufficient to observe long-term outcomes.

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which is associated with many complications and accounts for a third of all deaths globally.[4–6] CAD is predicted to continue to be the greatest and most widespread threat to human life through 2020;[7] it is also a multifactorial disease and affects complex interactions between physiological, genetic and lifestyle factors.[8]

Diet and lifestyle interventions can prevent atherogenesis, reduce the risk of cardiac events, and are viewed as an effective approach, mainly in patients with poor adherence to pharmacological therapies inherent in chronic diseases. In addition, nutritional interventions can also help lower public healthcare costs through preventive clinical interventions related to cardiovascular disease and reduce the burden on the government.[9 10] Vegetables are an important part of a healthy diet because they contain many bioactive components known as functional nutrients that have the benefits of promoting and maintaining human health. A strong document proposes that nitrate (NO_3), which exists in leafy green vegetables, beetroot, after reduction to nitrite (NO_2) is implicated in well-documented cardiac protection as it is a physiological substrate for nitric oxide (NO) production by the enterosalivary NO_3–NO_2/

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NO pathway.[11] Beetroot is a functional food that consists of various bioactive compounds such as nitrates, phenolics, ascorbic acid, carotenoids and betalains, and acts synergistically when consumed together.[12–14] As mentioned earlier, the cardiovascular health benefits of beetroot are attributed to its high concentration of inorganic nitrate.[15] For the first time, Webb *et al* investigated the effects of beetroot juice on endothelial function using the flow-mediated dilatation (FMD) technique in healthy volunteers. They reported an improvement in FMD technique at the end of the study.[16] Several studies have expanded this finding and examined the acute or chronic effects of nitrate on cardiovascular health.[17–21] Various studies, including systematic reviews and meta-analyses, have examined the effects of inorganic nitrate from beetroot on various risk factors for cardiovascular disease.[22–25] In a study by Nyberg *et al*, supplementation with red beetroot juice reduced muscle oxygen uptake and regulated blood flow.[26] Additionally, another study that examined the pharmacokinetic effects of red beet showed the beneficial effects of this supplement due to its high levels of nitrate and nitrite on lowering mean arterial pressure.[27] On the other hand, the effects of vitamin C on health, especially on cardiovascular disease prevention and amelioration, have been assessed in previous studies.[28] Documented studies show that vitamin C can improve cardiovascular function through various mechanisms; for example, due to the NO synthesis-enhancing ability of vitamin C and the NO-producing ability of inorganic nitrate of beetroot, there is a hypothesis that vitamin C with beetroot supplementation has a synergistic effect on the improvement of cardiovascular function.[29]

Despite the literature indicating a clear beneficial effect of inorganic nitrate intake and vitamin C supplementation for improving cardiovascular disease (CVD) risk factors, increased clinical trial investigation is required to understand its role in single and combined supplementation in improving cardiovascular function. Therefore, this study aimed to evaluate the effect of beetroot inorganic nitrate with or without vitamin C on endothelial function and arterial stiffness in patients with CAD.

METHODS AND ANALYSIS

The protocol for the current double-blind, randomised, placebo-controlled, parallel study follows the Consolidated Standards of Reporting Trials Standard Protocol Items: Recommendations for Interventional Trials guidelines and was approved by the Medical Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS. MEDICAL.REC.1399.717) and registered at the Iranian Registry of Clinical Trials (IRCT20210217050393N1; registered 16 May 2021). The recruitment and details of the current study protocol are shown in figure 1.

Recruitment

Recruitment will be performed at the cardiac outpatient clinic and Imam Reza Hospital related to Mashhad
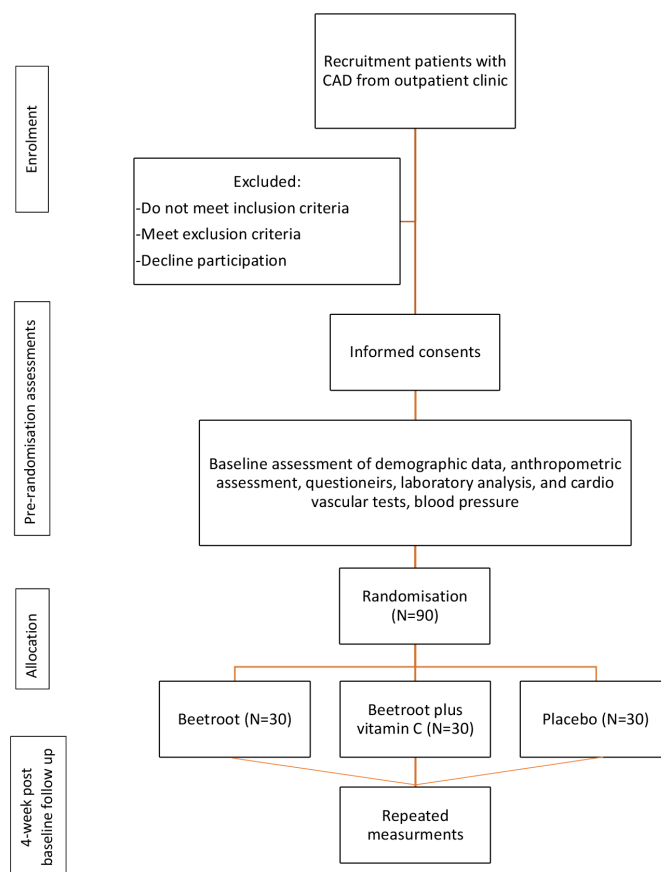
Figure 1 Trial procedure flow chart. CAD, coronary artery disease.

University of Medical Sciences, Mashhad, Iran. Participants will be selected for inclusion and exclusion criteria.

Eligibility

Individuals who are interested in participating will be assessed for eligibility criteria. They will then be offered the consent form to complete (online supplemental file 1).

Inclusion criteria

► Diagnosis of CAD by a cardiologist based on clinical criteria.
► Men and women in the age range of 30–65 years.
► Willingness to participate in research

Exclusion criteria

► History of chronic diseases (cancer, liver, kidney, or gall bladder abnormalities or disease)
► Acute heart attacks in the past year
► Take any medication outside the treatment protocol
► Nitrate-containing medications (such as nitroglycerin and isosorbide)
► Use of oral and/or injectable nutritional supplements for at least the last 4 months (vitamins D, C, E, calcium, magnesium, potassium and multivitamin-mineral, omega 3)
► Take any herbal medicine
► Allergy to red beets

Table 1 Timeline and tests

Time point	Weeks	
	0	4
Enrolment		
Eligibility screening	*	
Informed consent	*	
Randomisation	*	
Allocation	*	
Intervention		
Beetroot capsule: 3×500 mg	◄─────────►	
Beetroot+vitamin C capsule: 3×500 mg	◄─────────►	
Placebo: 3×500 mg	◄─────────►	
Assessments		
Laboratory: IL-6, hs-CRP, I-CAM, V-CAM, lipid profile, serum nitrate	*	*
Cardiopulmonary tests: PWV, AIX, heart rate, VO₂max, BP	*	*
Anthropometrics: height, weight, ABSI, FM, FFM, WC, HC and NC	*	*
Questionnaires: IPAQ, 72 hours food recall, SF-36	*	*
Side effects	◄─────────►	

ABSI, A Body Shape Index ; AIX, Augmentation Index; BP, blood pressure; FFM, fat-free mass; FM, fat mass; HC, hip circumference; hs-CRP, high sensitivity C reactive protein; I-CAM, intercellular adhesion molecule; IL-6, interleukin 6 ; IPAQ, International Physical Activity Questionnaire; NC, neck circumference; PWV, pulse wave velocity; SF-36, Short Form Health Survey; V-CAM, vascular cell adhesion molecule; WC, waist circumference.

► Pregnancy or lactation
► Addiction, alcoholism
► Do not consume more than 10% of capsules
► The occurrence of any accident that affects a person's health
► Having an acute illness during the study
► Reluctance to continue collaborating in research.

Study design and setting
We will include 90 patients with CAD as diagnosed by a cardiologist. Patients will receive 500 mg of beetroot capsules or beetroot capsules plus vitamin C or placebo three times/day for 4 weeks in equal ratios through a random assignment method (table 1). Beetroot capsules will contain 500 mg beetroot powders produced by the freeze-dried method, with the natural ingredients inorganic nitrate (3.2%), phenolics, ascorbic acid, carotenoids and betalains; and 100 mg vitamin C will be added to a third of the capsules for the beetroot plus vitamin C group, without any additives and preservatives.

Power calculation and sample size estimate
The study sample size was evaluated based on the Augmentation Index (AIX) from the Velmurugan et al study, as a primary outcome with an effect size of 0.8.[20] Type 1 and 2 errors were considered to be 5% and 20%, respectively (α=0.05 and β=0.2), with 80% power and 10% dropout. Finally, 30 participants were considered for each group.

Randomisation and blinding
Participants will be divided into three groups randomly based on the block randomisation method with an online database for clinical trials (www.sealedenvelope.com) consisting of four participants per block, which will be completed based on body mass index (BMI) (<30 and >30), diabetes (yes or no) and gender (male or female). For blinding in the randomisation process, unique codes will be used on the supplement boxes, and the desired code will also be generated online. The investigators and participants will remain blinded until the end of the study period. Additionally, the placebo capsules are in the same frame, taste and colour as the supplements, for concealment.

Safety
According to previous studies, except for limited gastrointestinal side effects (including nausea and vomiting) in a small number of people (approximately 9%), no side effects have been seen at this dose of the supplement. To ensure the health of the participants, the telephone of the facilitator and the doctoral student will be provided to the patients. In case of any problems or questions, quick contact is possible. Any possible complications will be recorded and will be decided in consultation with a cardiologist. The exchange of information and tracking of participants will be accelerated through social networks such as Telegram and WhatsApp.

Study assessments

All parameters will be assessed for all participants at the beginning and end of the 4-week study. A questionnaire of general information, the International Physical Activity Questionnaire, 72 hours food recall, and a quality of life and health questionnaire (Short Form Health Survey) will be completed for each individual. A complete list of foods containing high amounts of nitrate will be given to the participants, and they will be asked not to consume these foods during the study.

Anthropometric measurements

Height will be assessed on a flat surface without shoes, with a stadiometer. Weight, fat mass (FM) and fat-free mass (FFM) will be measured using a bioelectrical impedance analyser. Waist, hip and neck circumferences will be measured using a non-elastic metre tape. A Body Shape Index (ABSI) will also be measured using the statistical formula waist circumference (WC)/ $(BMI^{2/3} * Height^{1/2})$.

Device base parameters

Pulse wave velocity (PWV), AIX and heart rate will be measured using SphygmoCor. VO_2max/VO_2 peak, peak heart rate with cardiopulmonary cycle ergometer will be evaluated using a respiratory gas analyser (model: MetaLyzer3B, Cortex BioPhysic, Germany). Participants will be encouraged to continue pedalling until exhaustion and/or other stopping criteria, such as shortness of breath, leg fatigue and chest pain. Blood pressure will be measured with a mercury manometer three times at 5 min intervals in seated position with both feet flat on the floor after 10 min rest.

Laboratory analysis

To assess the serum level of some parameters in the fasting state, 10 mL of venous blood sample will be taken. The serum will be separated from blood samples by 3000 g centrifugation at 25°C and kept at −80°C until analysis. Interleukin 6 (IL-6), high-sensitivity C-reactive protein (hs-CRP), intercellular adhesion molecule (I-CAM) and vascular cell adhesion molecule (V-CAM) will be assessed using an ELISA kit. Nitrate will be measured by high-performance liquid chromatography. Additionally, lipid profile factors (triglycerides, total cholesterol and high-density lipoprotein cholesterol) will be measured using a biotecnica (BT) 1500 autoanalyser.

Statistical analysis

The analysis will be conducted based on the intention-to-treat test. Data will be analysed by using SPSS software, V.23 (SPSS, Chicago, Illinois, USA). Quantitative data will be presented as the mean±SD, and qualitative data will be demonstrated as frequencies and percentages. To determine the normality outcomes, the Kolmogorov–Smirnov test will be performed. One-way analysis of variance and/or the Kruskal–Wallis test will be used to compare the mean between the three independent groups. Covariance analysis will be used to detect any differences between the two treatment groups after adjustment for confounders. The results will be found to be statistically significant at a value p<0.05.

Primary outcomes

The primary outcomes of this study will be obtained by comparing the changes between and within groups of PWV, AIX, HR, systolic blood pressure (SBP), diastolic blood pressure (DBP), VO2max, and serum level changes of IL-6, hs-CRP, I-CAM, V-CAM, and lipid profile.

Secondary outcomes

Secondary outcomes will be obtained by comparing the changes between and within the groups after 4 weeks. These include anthropometric parameters such as height, weight, ABSI, fat mass (FM), fat-free mass (FFM), WC, hip circumference, neck circumference, quality of life and health.

Patient and public involvement

Patients and the public were not involved in the design, conduct, reporting, or dissemination of our research.

ETHICS AND DISSEMINATION

This randomised clinical study was approved by the Ethics Committee of Mashhad University of Medical Sciences (IR. MUMS.MEDICAL.REC.1399.717). All participants will be asked to complete the consent form at the beginning of the study. The results will be actively disseminated through peer-reviewed journals and conference presentations.

DISCUSSION

CAD is also called atherosclerosis or coronary heart disease, and it has been found to be the main cause of death and disability in humans. Epidemiological studies demonstrate that CAD is prevalent in almost all regions of the world. Various types of studies, such as case-control, epidemiological, clinical trials, systematic and meta-analyses, have been conducted to understand the various aspects of this disease. Understanding the relevant risk factors, prevention strategies and early treatment are essential for the management of such CAD. Many facts and data have been published describing the current methods of preventing and treating CAD, but there is still a lack of awareness that makes it difficult to manage it. Results from previous studies have shown the effectiveness of inorganic nitrate of beetroot in the prevention of cardiovascular disease, especially atherosclerosis such as CAD, because of NO production in the body that prevents the atherosclerosis process. But there is still a lack of knowledge about the treatment of CAD that makes this trial necessary. Although the follow-up period is not sufficient to observe long-term outcomes, based on previous studies, it is expected that the inorganic nitrate of beetroot will improve cardiovascular function and health status of patients with CAD and its effect may be enhanced with vitamin C supplementation.

Trial status

The trial enrolment started in November 2021 and is currently recruiting patients. Follow-up and collection of labour data of patients are expected to take time, approximately until June 2022.

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