Beetroot Juice Supplementation Increases High Density Lipoprotein-Cholesterol and Reduces Oxidative Stress in Physically Active Individuals

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Abstract: Beetroot juice contains a high level of biologically accessible antioxidants, beneficial phytochemicals and dietary nitrate, which seem to exert beneficial effects in human health. Dietary nitrate, from beetroot has been reported to lower blood pressure. However the impact of beetroot on lipid profile and oxidative stress is unknown. In present study, the effect of supplementation with beetroot juice for 15 days was investigated. Plasma lipid profile, antioxidant status, oxidative stress and body composition changes were evaluated at baseline and after 15 days of beetroot juice supplementation. Beetroot juice supplementation beneficially influenced the lipid profile by significantly increasing the levels of high-density lipoprotein cholesterol (HDL-C) from 42.9 ± 8.3 mg/dl to 50.2 ± 9.8 mg/dl and decreasing low-density lipoprotein cholesterol (LDL-C) from 129.7 ± 82.3 mg/dl to 119.5 ± 79.2 mg/dl compared with baseline values. Beetroot juice supplementation increased ($P < 0.05$) plasma nitrite level and guanosine 3', 5'-cyclic monophosphate (c-GMP) levels. A significant increase in plasma total antioxidant capacity and vitamin C levels was observed after beetroot juice intake for 15 days. There was no significant change in the body fat mass and lean body mass of participants with the beetroot juice supplementation. Beetroot juice supplementation significantly decreased the stress markers plasma hydroperoxides and cortisol levels. Beetroot juice acts as a potent vasodilator by increasing plasma c-GMP levels and nitrite levels. Beetroot juice consumption improves plasma lipid profile and antioxidant status, encouraging further evaluation on a population with higher cardiovascular disease risk.

Keywords: Antioxidants, Lipid profile, Cortisol, Supplementation, Cardiovascular health.

INTRODUCTION

The health benefits of consuming fruits and vegetables, as seen with the DASH (Dietary Approaches to Stop Hypertension) diet, have generated significant interest over the years, especially in certain sectors of the population looking for alternative approaches to conventional medical treatment. Large scale longitudinal observational studies exhibited the associations between fruit and vegetable intake and reductions in risk for cardiovascular disease [1-5]. While these benefits were initially linked with the rich source of micronutrients such as vitamins and minerals found in these foods, subsequent studies utilizing supplements with these nutrients failed to provide the same effect as intake of the foods [6].

Now days, nitrate is one of the popular natural chemicals found in many of vegetables that has considerable role in treating a variety of diseases associated with poor vascular health. Dietary nitrate is converted to nitrite in the body and thereafter to nitric oxide (NO). Nitrate has been shown to improve endothelial function, reduce blood pressure and the oxygen cost of sub-maximal exercise, and increase regional perfusion in the brain.

In humans, it is reported that nitrite represented a major bioavailable pool of Nitric Oxide (NO), which caused a rapid formation of erythrocyte iron-nitrosylated hemoglobin and, to a lesser extent, *S*-nitroso-hemoglobin [7]. NO-modified hemoglobin formation was inversely proportional to oxyhemoglobin saturation, potentially contributing to hypoxic vasodilation.

The beneficial effects of dietary nitrates on delaying the development and treatment of prehypertension and hypertension were reported as the supplementation with an acute dietary nitrate load caused effects that correlate with a rise in circulating levels of nitrite derived from dietary nitrate, like a marked reduction in blood pressure (BP) in normotensives, reduced platelet aggregation, and protection against experimentally-induced endothelial ischemia/ reperfusion (I/R) injury [8].

The most abundant supply of dietary nitrate is found in beetroot (*Beta vulgaris*). Drinking beetroot juice (BRJ) provides a more convenient alternative to consuming the whole vegetable. Beetroot juice

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contains a high level of biologically accessible antioxidants [9], a number of phenolic compounds [10,11] and a nitrogen containing pigments called betalains, which are composed of betacyanins that confer the red-violet color to beetroot and a yellow-orange colorant betaxhantins [11]. It also contains other health promoting compounds such as potassium, magnesium, folic acid, iron, zinc, calcium, phosphorus, sodium, niacin, biotin, vitamin B6 and soluble fibre [12]. Beetroot juice has more than 12 times higher nitrate content than other vegetable juices.

Beetroot juice has been used in numerous scientific studies in the last several years, primarily involving aspects of its role in vascular health. Dietary nitrate found in our foods is taken up in the upper gastrointestinal tract and gets mixed in the blood with nitrate formed endogenously by dioxygenation of nitric oxide; nitrate has a plasma half-life of 5-6 hours. The nitrate to nitrite cycle leads to fairly long term enhanced levels of plasma nitrite. Up to 25% of plasma nitrate is taken up by salivary glands so that concentrations of nitrate in saliva can be 20-fold higher than in plasma [13]. Part of this nitrate is converted to plasma nitrite by the action of oral bacteria. Other fruit juices like pomegranate juice, cranberry juice and acai juice are also rich in nitrate and nitrites. Hot dogs, ham, pork tenderloin, bacon, and nitrate- or nitrite-free bacon had high nitrate and nitrite concentrations [14].

While improvements in performance of both aerobic and anaerobic exercise are reported *via* numerous proposed mechanisms, the impact of BRJ serving as a potent dietary antioxidant need to be explored.

So keeping these points in mind, in the present study, we examined the effects of 15 days dietary nitrate supplementation on blood pressure, heart rate, and biochemical parameters such as total antioxidants, nitrite, vitamin C, the markers of cardiovascular risk such as lipid profile, as well as and oxidative stress marker i.e. hydroperoxides and cortisol in a group of physically active individuals.

MATERIALS AND METHODS

Plan of Study

A group of randomly selected 30 infantry soldiers participated in the study. Their age, physical variables and dietary habits are listed in Table **1**. Participants were explained about the experimental procedures and gave written informed consent before participation in the study. Soldiers were instructed to arrive at the laboratory in morning hours after overnight fast and to avoid strenuous exercise in the 24 h preceding each testing session. Subjects were instructed to avoid foods rich in nitrate (such as leafy green vegetables and processed meats) during the study period.

Table 1: Physiological Parameters of Soldiers (n=30)

Age (Yrs)	29.9±_4.2
Height (cm)	171.9 ± 3.9
Weight (Kg)	72.1 ± 7.8
BP (Sys) (mm Hg)	125.2 ± 12.1
BP (Dia) (mm Hg)	66.9 ± 9.2
HR (Beats/ min)	71.9 ± 10.1

*The values are Mean ± SD of thirty individuals.

The beetroot juice was prepared and packed at Defence food research laboratory (DFRL), Mysore, India. After collection of basal data participants were supplemented with 400ml beetroot juice (consumed twice daily) for 15 days. All measurements were repeated after 15 days of supplementation.

Physiological Variables and Body Composition

Body composition of participants, at baseline and after 15 days of supplementation, was analyzed using a bioelectrical impedance based body composition analyzer (Tanita, TBF-310GS). Body composition parameters included body weight (Kg), body fat (%), fat mass (kg), body mass index BMI (Kg / m^2), basal metabolic rate BMR (Kcal), lean body mass (Kg) and total body water (Kg).

Biochemical Variables

The blood sample (~ 5.0 ml) of subjects after overnight fast were taken in EDTA *(*ethylene di amine tetra acetic acid) tubes. Blood samples were centrifuged immediately at 2000x*g* for 10 minutes at 4°C to recover plasma and stored at -80°C until assayed. Aliquot of whole blood, whole blood samples mixed with equal volume of 10% meta phosphoric acid and separated RBC(s) were stored at -80° C until analyzed for biochemical parameters.

Hemoglobin concentration in whole blood was analysed using cyanmethemoglobin method, plasma c-GMP and plasma Cortisol levels were analyzed using kits supplied by Drg International Inc. USA and XEMA co. ltd, Germany, respectively (cGMP direct EIA3069 and cortisol EIA K 210). The total plasma antioxidant status was measured using an $ABTS^{\cdot}$ Radical cation decolorization assay [15]. This assay was based on the inhibition by antioxidants of the free radical cation from ABTS (2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid diamonium salt) (Sigma-Aldrich, USA). ABTS was incubated with potassium persulfate (Sigma-Aldrich, USA) in order to produce a free radical cation ($ABTS^{o\pm}$). This had a relatively stable blue-green color, which was measured by spectrophotometer at 734 nm. This assay was calibrated using Trolox, a water-soluble vitamin E analog (Aldrich Chemical, USA) as standard. Plasma nitrite levels were estimated by using Greiss reagent [equal mixture of 1% sulphanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine hydrochloride in distilled water] and the optical density was measured at 540 nm spectrophotometrically. Plasma Vitamin C levels were analyzed using αα'- dipyridyl method [16]. Plasma hydroperoxides were estimated using FOX-1 assay [17]. Total plasma protein levels were estimated by Lowry method [18].

The lipid profile studied included total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). The concentration of total cholesterol, HDL-C and TG were assayed using kits supplied by RANDOX laboratory, USA. LDL-C was calculated by the formula of Friedewald [19].

Statistical Analysis

The Graph Pad Prism Software (version 5) was used for data analysis. All data are expressed as mean ± SD unless otherwise stated. Data were analyzed by t-test for comparison with the baseline or control and after supplementation data. Differences were considered significant if $P < 0.05$.

RESULTS

There was no significant differences in the physiological parameters of physically active individuals (n=30) who participated in the study (Table **1**). The mean age and height of the soldiers participated in the study was 29.9 ± 4.2 years and 171.9 ± 3.92 cm respectively. Beetroot juice was generally well tolerated by the subjects. Common effects, such as beeturia (red urine) and red stools, of beetroot juice were observed as expected. The nitrate concentration in the beetroot juice was 3.3 mM. The range of betalin concentration in beetroot juice was observed as 35-70 mg.

Effect of Supplementation on Body Composition

The change in body composition parameters are depicted in Table **2**. There was no change in the body weight, body resistance, basal metabolic rate and body water of soldiers. A non-significant ($P > 0.05$) decrease in the percent fat mass, kilogram fat mass and body mass index, from 17.8 ± 4.4 % to 17.5 ± 4.1 % ; 12.8 ± 4.4 Kg to 12.7 ± 4.0 Kg and 24.1 ± 2.5 Kg/m^2 to 24.1 ± 2.4 Kg/m^2 , respectively was observed. There was non-significant increase ($P > 0.05$) in lean body mass from 57.9 ± 3.9 Kg to 58.1 ± 4.3 Kg was observed.

Effect of Supplementation on Hemoglobin and Antioxidant Status

Upon beetroot juice supplementation, a non-significant ($P > 0.05$) increase in the hemoglobin levels from 15.11 ± 1.66 g/ dl to 15.96 ± 1.86 g/ dl and a significant increase ($P < 0.05$) in plasma total antioxidants levels, from 1.0 ± 0.11 mM to 1.2 ± 0.12 mM, were observed (Table **3**).

Table 2: Effect of Beetroot Juice Supplementation on Physiological Parameters

	Control	Beetroot juice supplemented
Body Weight (Kg)	70.86 ± 7.41	70.74 ± 7.09
Body Fat (%)	17.86 ± 4.44	17.55 ± 4.15
Body fat mass (Kg)	12.81 ± 4.44	12.75 ± 4.04
Body Mass Index (Kg/m^2)	24.19 ± 2.57	24.07 ± 2.40
Body Resistance (Ω)	511.96 ± 40.93	509.32 ± 63.22
Basal Metabolic Rate (Kcal)	1676.28 ± 145.75	1699.72 ± 102.69
Lean Body Mass (Kg)	57.92 ± 3.96	58.10 ± 4.31
Body water (Kg)	42.38 ± 2.88	42.53 ± 3.17

*The values are Mean ± SD of thirty individuals.

Table 3: Effect of Beetroot Juice Supplementation on Biochemical Parameters

	Basal	Supplemented
Hb (g/dL)	15.11 ± 1.6	15.96 ± 1.8
Total Antioxidants (mM)	1.0 ± 0.11	1.2 ± 0.12
Plasma Nitrite (µmoles/l)	5.07 ± 0.07	6.79 ± 0.14
Plasma c-GMP (nmol/l)	9.4 ± 6.7	13.8 ± 7.0
Total cholesterol (mg/dL)	197.54 ± 96.7	193.49 ± 92.4
HDL-C (mg/dL)	42.92 ± 8.39	50.23 ± 9.8
LDL-C (mg/dL)	129.70 ± 82.3	119.50 ± 79.2
Triglycerdes (mg/dL)	124.50 ± 23.9	118.91 ± 39.3
Hydroperoxides (µ mole /ml)	10.11 ± 2.01	8.02 ± 2.26
Plasma Cortisol (nmol/l)	305.4 ± 90.0	238.5 ± 78.2
Plasma Vitamin C (mg/dl)	1.6 ± 0.8	1.8 ± 0.3
Total Protein (g/ml)	7.5 ± 1.2	7.3 ± 1.1

*The values are Mean ± SD of thirty individuals.

Effect of Supplementation on Plasma Nitrite and Plasma c-GMP Concentrations

Beetroot juice supplementation significantly increased ($P < 0.05$) plasma nitrite level from 5.07 ± 0.07 µmoles/l to 6.79 ± 0.14 µmoles/l. It is due to the reason that the beetroot juice is rich in nitrite and nitrate levels. A significant increase ($P < 0.05$) in the Guanosine 3', 5'-cyclic monophosphate (c-GMP) levels from 9.4 ± 6.7 nmol/l to 13.8 ± 7.0 nmol/l was observed in the beetroot juice (BRJ) supplementation plasma samples in comparison to the baseline plasma samples (Table **3**).

Effect of Supplementation on Lipid Profile (Total Cholesterol, HDL-C, LDL-C, Triglycerides)

In this study, beetroot juice supplementation beneficially influenced the lipid profile by significantly increasing the beneficial cholesterol i.e., high-density lipoprotein cholesterol (HDL-C) levels from 42.9 ± 8.3 mg/dl to 50.2 ± 9.8 mg/dl and decreasing ($P< 0.05$) low density lipoprotein cholesterol (LDL-C) from 129.7 ± 82.3 mg/dl to 119.5 ± 79.2 mg/dl compared with baseline period, while total cholesterol and triglyceride levels decreased non significantly (Table **3**).

Effect of Supplementation on Stress Markers and Plasma Vitamin C

The plasma hydroperoxides and cortisol levels were decreased significantly. A significant decrease in plasma hydroperoxide and cortisol levels were observed from 10.1 ± 2.0 to 8.0 ± 2.3 µ mole /ml and from 305.4 ± 90.0 nmol/l to 238.5 ± 78.2 nmol/l, respectively upon supplementation. Whereas there was a significant increase ($P < 0.05$) in the plasma vitamin C level from 1.6 ± 0.8 mg/dl to 1.8 ± 0.3 mg/dl (Table **3**). There was a non-significant change in total plasma protein (Table **3**).

DISCUSSION

Findings suggest the beneficial effects of dietary nitrate rich beetroot juice. There was no significant change in the body weight, basal metabolic rate, body water but a decrease in fat mass and body mass index of soldiers.

Beetroot juice supplementation significantly increased ($P < 0.05$) plasma nitrite level from 5.07 ± 0.071 µmoles/l to 6.79 ± 0.148µmoles/l. It is due to the reason that the beetroot juice is rich in nitrite and nitrate levels. Similarly, some studies also reported that the plasma nitrite and nitrate levels reached a peak at 3 hours and remain elevated at 24 hours upon beetroot juice administration to the body. The beetroot juice nitrate converts into nitric oxide which caused decrease in systolic BP and no change in diastolic BP at 24 hours [20,21 and 22]. A significant increase in the Guanosine 3', 5'-cyclic monophosphate (c-GMP) levels observed in the beetroot juice supplemented plasma samples in comparison to the baseline plasma samples. Researchers have reported cGMP as the most sensitive indicator of NO bioactivity and an increase in its concentration provides unequivocal evidence of the production of bioactive NO. In the vascular wall, the initiation of cyclic GMP–mediated intracellular signals causes the biological effects of nitric oxide [23]. Loss of nitric oxide (NO) bioactivity, leading to deficient soluble guanylatecyclase (sGC) activation and cGMP production, underpins many of the hemodynamic and morphological changes in the cardiopulmonary circulation that characterize hypertension, particularly pulmonary arterial hypertension. Beetroot juice supplementation is a therapeutic approach that is NO dependent signaling, causing c-GMP production that is clinically effective in patients with the disease [20].

Cardiovascular disease (CVD) is the number one cause of mortality globally [21]. Epidemiological evidence suggests that increased consumption of vegetables reduces the risk of CVD [26-29]. In our study, beetroot juice supplementation beneficially influenced the lipid profile by significantly increasing the beneficial cholesterol i.e., high-density lipoprotein cholesterol (HDL-C) levels and decreasing low density lipoprotein cholesterol (LDL-C) in comparison with baseline period, while total cholesterol and triglyceride levels decreased non significantly. These changes in lipid profile could be because of the Betalains, which is an important phyto-chemical present in beetroot juice, that have contributed to the cardiovascular benefits as these are nitrogen-containing color compounds. This finding is supported by a 2 week of cactus pear fruit, containing 10 mg/100 g betalains, supplementation study. The study cleared the role of Betalains in oxidative stress-related diseases such as CVD [30].

The cells continue to produce reactive oxygen species (ROS) in normal metabolic processes and through a defence mechanism of antioxidants and antioxidant enzymes, these cells also protect themselves from the toxicity of ROS [31]. But an imbalance between the production of ROS and neutralization by antioxidant defence system, the oxidative stress is developed and increased levels of stress markers [32]. It was clear from the observations that upon 15 days beetroot juice supplemention, there was significant decrease in plasma hydroperoxides as well as plasma cortisol levels. An increased plasma vitamin C levels acts as a good non enzymatic antioxidant which leads to decreased reactive oxygen species and finally could be the reason of decreased hydroperoxides levels. In support to our findings, interestingly, several recent investigations have examined the potential antiradical properties of betacyanins and betaxanthins, the main pigments of red beetroots [33]. Beetroot juice is a rich source of bioactive components such as β-carotene, α-tocopherol, betanin, and polyphenols, and these compounds have highly effective antioxidant properties. It is earlier reported that the beetroot juice remarkably reduced hydroperoxides in plasma and showed positive antioxidative effects. Similarly, lyophilized apple supplementation [34] and freeze-dried red beet leaf supplementation [35] resulted in a reduction of lipid peroxidation in rats fed cholesterol-enhanced diet, suggesting that vegetable intake can provide a protection against oxidative stress. In addition, it is earlier reported that linoleate peroxidation by cytochrome c was inhibited by betanin from red beets. It was suggested that regular beetroot consumption may provide protection against certain oxidative stress-related disorders in humans [36]

An adequate nutritional support is must for human in order to maintain the highest level of physical fitness under different climatic conditions. So our results demonstrated that a regular intake of beetroot juice is a better way to increase antioxidant protection and improvement of lipid profile. It highlights the potential of a “natural” low cost approach for the treatment of cardiovascular disease and for effective performance enhancement at extreme environment conditions.

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