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Standardized Beetroot Extract in Cardiovascular and Exercise Performance: A Randomized, Double Blind, Placebo Controlled, Crossover, Two Group, Two Periods, Clinical Study to Evaluate the Efficacy and Safety

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ABSTRACT:

Increased plasma nitrate concentrations from dietary sources of nitrate have proven to benefit cardio vascular and exercise performance. Beetroot (BR) contains relatively high levels of nitrate (NO_3), which increases nitric oxide storage. This study investigated whether dietary nitrate supplementation, in the form of a standardized beetroot extract formulation drink, could improve cardio vascular and exercise performance. Limited data is available regarding the effect of nitrate ingestion on cardio vascular and exercise performance, and limited studies have investigated the potential ergogenic effects of a small-volume, concentrated dose of beetroot juice. In a randomized double blind, placebo controlled, cross over, two groups, two periods design, 20 male healthy volunteers aged between 18-55 years were instructed to take one sachet per day (either Sabeet® (5.0 grams of Sabeet® sachet contains standardized beetroot extract with 2% nitrates) or Placebo before their daily exercise bout for a period of 14 days in Period I. After a washout period of 7 days, the treatment was crossed over for Period II, wherein subjects who received active product in period I received placebo in Period II which was again for 14 days. Present study demonstrates that dietary NO_3^-, administered in the form of beetroot juice, decreases systolic blood pressure (SBP) and improves cardiac output. These results may provide a mechanism by which nitrate exerts beneficial effects on muscle function with applications in sports performance.

Keywords: *- Beetroot, Blood Pressure, heart rate, stroke volume and cardiac output.*

INTRODUCTION

In the world of athletic competition, margins of victory are becoming smaller, and in some cases may literally come down to a fraction of a second or the ability to contract a single motor unit one more time. The forecast for "Estimated Compound Annual Sales Growth" from 2010 to 2017 is projected to be 5% for supplements compared to 8% for natural and organic foods [1].

NITRATE AS A PERFORMANCE ENHANCER

Nitrate has received considerable attention in recent years and is quickly gaining traction as a health and performance enhancing nutritional supplement. Once ingested, inorganic NO_3 metabolizes in vivo to bioactive nitrite (NO_2) and is subsequently salvaged and circulated inhuman blood. Nitrate (NO_3) is a naturally occurring anion in the human body, initially believed to be an inert by product of nitric oxide metabolism. NO_2^- exerts these effects in the body via its conversion to functional nitrogen oxides (NO_x), including nitric oxide (NO). Bacteria located in the oral cavity are integral in bioactivating and reducing NO_3 to NO_2, through an enzymatic reduction process now commonly referred to as the entero salivary pathway [2].

Numerous studies have now confirmed the vasodilating effects of low-dose nitrite in mice, rats, sheep, dogs, primates and humans [2-8]. Dietary supplementation with inorganic nitrate (NO_3^-), which undergoes a stepwise reduction to nitrite (NO_2^-) and then nitric oxide (NO) and other reactive nitrogen species, has been reported to reduce the O_2 cost of sub maximal exercise [9-14].

Nitrate and nitrite play a key role in modulating blood pressure in both healthy and disease states. The vast arrays of surprisingly diverse mechanisms by which NO can be generated from nitrite, serve to underline its vital role in

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regulating vascular tone. Its physiological handling appears to be designed to deliver NO to the point of greatest need with evidence that nitrite reduction increases with increasing hypoxia and falling pH-an effect perhaps most evident in certain disease states. In addition, evidence is emerging that nitrite may have a direct vasoactive effect independent of its role as a precursor for NO [15].

Many authors have reported that NO_3^- supplementation (mainly administered via non standardized beetroot juice) can improve finish times [13,16], elongate time to exhaustion [10,17-19], reduce the gross O_2 cost of exercise [10,11,18,20,21], and increase peak power [13,22,23] and work rate [10,12,20,23]. With regard to the above, it should also be noted thatother authors have also reported no ergogenic effect of NO_3^- supplementation [24-27]. Many of these studies have also evaluated cycling exercise performed by well-trained or elite-level athletes to improve due to intake of dietary nitrates [1].

Beetroot is a rich source of dietary NO_3^- [28] and a number of studies have investigated its potential for reducing blood pressure in humans [10,21,29-31], which appears to be more potent in men [32].

Beetroot is as an exceptionally rich source of antioxidant compounds. The beta lain pigment in particular, has been shown through several *in vitro* studies to protect cellular componentsfrom oxidative injury [33-35]. Based on the available data, beetroot appears to be a powerful dietary source of health promoting agents that holds potential as therapeutic treatment for several pathological disorders. The powerful antioxidant, anti-inflammatory and vascular-protective effects offered by beetroot and its constituents have been clearly demonstrated by several *in vitro* and *in vivo* human and animal studies; hence its increasing popularity as a nutritional approach to help manage cardiovascular disease and cancer. In the human studies to date, beetroot supplementation has been reported to reduce blood pressure, attenuate inflammation, avert oxidative stress, preserve endothelial function and restore cerebrovascular hemodynamic. Furthermore, several studies have now established beetroot supplementation as an effective means of enhancing athletic performance [11, 20, and 36].

MATERIAL & METHODS

STUDY DESIGN:

This was a randomized, double blind, placebo controlled, cross over, two groups and two periods study. Twenty healthy adult male subjects, physically active but not exercise trained, ranging in age from 18 to 55 years, normotensive, were enrolled. Subjects who were non-smokers, non-alcoholics and not using any concomitant medications including vitamins and minerals, during or before the course of the study as evidenced by written informed consent were included in the trial. Subjects were enrolled for two periods Period I and Period II. Eligible subjects were randomly assigned, in a 1:1 ratio, to receive either Sabeet® or placebo, respectively.

ETHICS AND INFORMED CONSENT

This trial was conducted in accordance with the clinical research guidelines established by the Drugs and Cosmetics Act, 1940 of India, Drugs and Cosmetics Rules, 1945 of India, Ethical Guidelines for Biomedical Research on Human Participants, 2006 of Indian Council of Medical Research (ICMR) in India, the principles enunciated in the Declaration of Helsinki (Edinburgh, 2000) and the ICH harmonized tripartite guideline regarding Good Clinical Practice (GCP). Written and oral information about the study in a language understandable by the subject was provided to all subjects. This study was registered at Clinical Trials Registry- India (www.ctri.nic.in) under the identifier CTRI/2015/11/006387 on 26 Nov 2015. There were no changes to the methods or planned endpoints after study initiation.

PARTICIPANTS

Twenty healthy males (10 pairs) participated in the study. Each pair consisted of individuals matched for age, height and weight. One member of each pair was randomly assigned to the treatment group receiving Sabeet® supplementation (n= 10), while their matched counterpart received placebo (n= 10) (Figure 1). Before taking part in the study, all participants read and signed an informed-consent form approved by Institutional Ethics Committee, Sparsh Hospital for Advanced Surgeries for Human Subjects.

Subjects were included in the study if indicated “Yes” to all of the inclusion criteria and “No” to all of the exclusion criteria.

Inclusion Criteria (1) Male healthy adult subjects ranging in age from 18 to 55 years (both inclusive), normotensive, physically active but not exercise trained. (2) Willingness to follow the protocol requirements as evidenced by written, informed consent. (3) Agree not to use any medication (prescription and over the counter), including vitamins and minerals, during or before the course of this study. (4) Subjects whose blood chemistries are within a normal range or not considered clinically significant if outside the normal range. (5) Non-smokers and non-alcoholics. (6) Willing to come for all follow-up visits.

Exclusion Criteria (1) Any clinically significant medical history, medical finding or an ongoing medical or psychiatric condition exists which in the opinion of the

Investigator could jeopardize the safety of the subject, impact validity of the study results or interfere with the completion of study according to the protocol. (2) Significant abnormal findings as determined by baseline history, physical examination, vital signs (blood pressure, pulse rate, respiration rate, temperature) hematology, serum chemistry. (3) History of hypersensitivity reactions. (4) Participation in a clinical study during the preceding 90 days. (5) Any contraindication to blood sampling. (6) Blood or blood products donated in the past 30 days prior to study supplement administration.

SABEET® SUPPLEMENTATION

Subjects were instructed to self–administer the study product every day for a period of 14 days. Each 5.0 grams of Sabeet® sachet contains beetroot extract standardized to 2% nitrates.

DATA REPORTING AND MANAGEMENT

All data was reported in the respective hospital records that were then transcribed onto the Case Report Forms (CRFs). All data reported was first reviewed by the respective investigators present at the three sites and then entered by the clinical research coordinators onto the CRFs. This data entered in the CRFs was again verified by the investigators a second time. It was ensured that the source data matches with the data entered in the CRFs complying with the GCP guidelines on source data verification. Data collection during this clinical study and preparation of the data for analysis was conducted by separate and independent functional groups. Standard procedures ensured all CRFs were tracked and properly routed. The training of all the end users and clinical data management associates pertaining to the database entry and validation process was documented. The data entry operator transcribed the data from the paper CRF to the database. Data validation was conducted by the data manager. The database was locked post reconciliation of all data. The locked database was provided to the statistician who was independent of the study team. The data was then analyzed statistically.

STATISTICAL ANALYSIS

Statistical Analysis Software (SAS) version 9.2 was used for data analysis here. Paired t-test, Analysis of Covariance (ANCOVA) and Wilcoxon signed rank sum test were used for appropriate data set variables to reach the best possible statistical conclusion between Sabeet® and Placebo receiving groups. The baseline descriptors were summarized as mean and standard deviations for continuous variables and as frequencies and percentages for categorical variables. Last Observation Carry Forward (LOCF) method was followed for efficacy evaluations of subjects, whose data was not available in the last visit.

SAFETY OUTCOMES

Safety of the study was assessed considering the occurrence of adverse events, safety blood parameters, and the follow-up of vital signs (blood pressure and heart rate). Laboratory data was summarized by presenting summary statistics of raw data and change from baseline values to end of study in laboratory values relative to normal reference limits. The four adverse events reported were mild in nature and was found to be ‘not related/unrelated’ to the study product. The reported adverse events were resolved without any treatment.

EFFICACY OUTCOMES

Efficacy assessments were standing blood pressure, heart rate, stroke volume and cardiac output, ESR and Hs-CRP, plasma nitric oxide (NO) levels, lactate dehydrogenase tests.

RESULTS

A total of 20 subjects were enrolled into the study. There were no subject withdrawals or dropouts during this study. The 20 subjects enrolled into the study were randomized into active and placebo groups in 1:1 ratio. None of the enrolled subjects had abnormal medical history.

Efficacy Evaluation:

The *‘p’* value suggests that there was a statistically significant improvement in the cardiovascular and exercise performance from baseline to final visit, between the placebo and Sabeet® arms. Statistical analysis using Analysis of Co-Variance (ANCOVA) showed the efficacy parameters were found to be statistically significant ($p < 0.05$) between the Sabeet® and placebo groups (Table 1-2). Furthermore, comparative mean values of efficacy assessments between Sabeet® and placebo groups across various visits are presented for efficacy parameters (Graph 1-4).

Graph 01: Effects of Sabeet® on Erythrocyte Sedimentation Rate. Bars represent means for Erythrocyte Sedimentation Rate levels, expressed in mg/L. The study subjects were 20 healthy, normotensive young adult males. Left panel: placebo Vs Sabeet® (pre exercise). Right panel: placebo Vs Sabeet® (post exercise).

∗Difference between placebo and Sabeet® statistically significant at $P < 0.05$.

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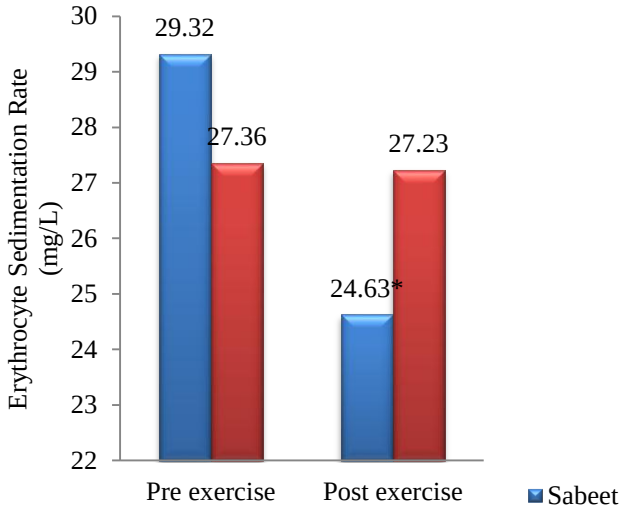
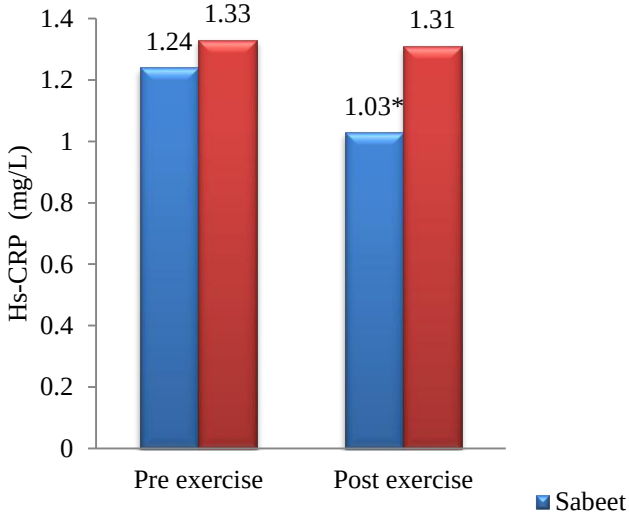


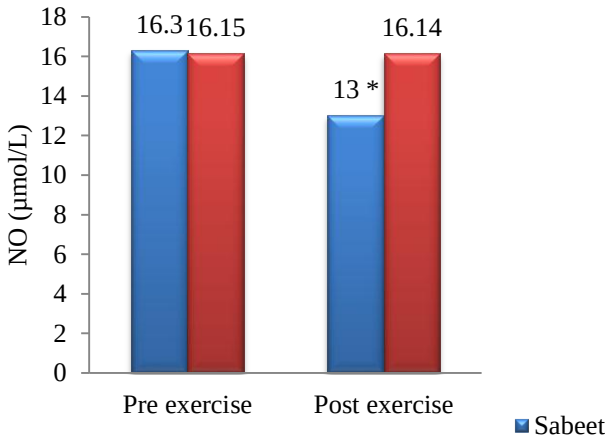
Graph 02: Effects of Sabeet® on Hs-CRP. Bars represent means for Hs-CRP levels, expressed in mg/L. The study subjects were 20 healthy, normotensive young adult males. Left panel: placebo Vs Sabeet® (pre exercise). Right panel: placebo Vs Sabeet® (post exercise).

∗Difference between placebo and Sabeet® statistically significant at $P< 0.05$.



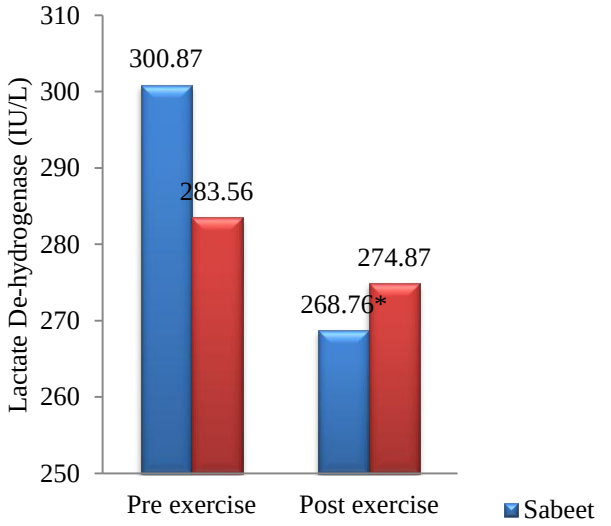
Graph 3: Effects of Sabeet® on Plasma NO levels. Bars represent means for Plasma NO levels, expressed in µmol/L. The study subjects were 20 healthy, normotensive young adult males. Left panel: placebo Vs Sabeet® (pre exercise). Right panel: placebo Vs Sabeet® (post exercise).

∗Difference between placebo and Sabeet® statistically significant at $P< 0.05$.



Graph 4: Effects of Sabeet® on plasma Lactate De-hydrogenase. Bars represent means for plasma Lactate De-hydrogenase levels, expressed in IU/L. The study subjects were 20 healthy, normotensive young adult males. Left panel: placebo Vs Sabeet® (pre exercise). Right panel: placebo Vs Sabeet® (post exercise).

∗Difference between placebo and Sabeet® statistically significant at $P< 0.05$.



Efficacy parameters like heart rate, stroke volume and cardiac output showed statistical significance between active and placebo groups **(Table 1)**.

Table 1: Efficacy analysis for Heart Rate, Stroke Volume and Cardiac Output

Parameter(Post Exercise)	Placebo	Sabeet®	*p-value*
Heart Rate (beats/min)	85.67 (24.00)	92.71 (25.57)	0.0083
Stroke Volume (ml/beat)	75.05 (11.25)	77.56 (11.53)	0.0886
Cardiac Output (L/min)	5.34 (0.78)	5.58 (0.80)	0.0048*

Values expressed as Mean (SD)
**p value significant (<0.05) between the treatment groups*

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Plasma [NO]: The Sabeet® group mean plasma [NO] values pre exercise was 16.30 (2.18) and post exercise was 13.00 (2.50). Relative to placebo, Sabeet® ingestion decreased plasma [NO] ($p<0.05$). These results indicate that the Sabeet® increases circulating nitric oxide and result in an improvement in blood flow, muscle "pump", and exercise performance.

Lactate Dehydrogenase (LDH): The Sabeet® supplementation significantly reduced LDH from pre exercise to post exercise ($p<0.05$). These results indicate that supplementary Sabeet® decreased serum concentrations of the intramuscular enzyme LDH following exercise, even when the recommended intake of Sabeet® was being consumed. This observation suggests that Sabeet® supplementation may reduce the muscle damage associated with endurance exercise.

Changes in inflammatory biomarkers (Erythrocyte Sedimentation Rate, C Reactive Protein): Inflammation is an intricate network of reactions; the level of Erythrocyte Sedimentation Rate (ESR) C-reactive protein (CRP) is considered one of the measures to provide an overall estimate of inflammatory status. CRP levels in the Sabeet® group was decreased significantly from 1.24 mg/L on pre exercise to 1.03 mg/L on post exercise ($p < 0.05$) and ESR levels in the Sabeet® group was decreased significantly from 29.32 mg/L on pre exercise to 24.63 mg/L on post exercise ($p < 0.05$) after consumption of the Sabeet®.

The significant decrease in CRP and ESR suggests that consumption of the Sabeet® might be able to lower the inflammatory status of healthy adults**(Table 2)**.

Table 2: Efficacy analysis for biomarkers

Parameter	Placebo		Sabeet®		*p-value*
	PRE-EXERCISE	POST- EXERCISE	PRE-EXERCISE	POST- EXERCISE	
ESR (mg/L)	27.36 (16.74)	27.23 (23.62)	29.32 (18.65)	24.63 (23.86)	0.0209*
hs-CRP (mg/L)	1.33 (0.25)	1.31 (0.31)	1.24 (0.32)	1.03 (0.31)	0.0177*
Plasma NO levels (µmol/l)	16.15 (2.56)	16.14 (2.48)	16.30 (2.18)	13.00 (2.50)	0.0363*
Lactate Dehydrogenase (U/L)	283.56 (72.58)	274.87 (75.89)	300.87 (66.75)	268.76 (61.11)	0.0189*

Values expressed as Mean (SD)
**p value significant (<0.05) between the treatment groups*

Graphical data presented for all these efficacy assessments is presented as (Graph 1 - 4) and it has been observed that overall, the group that received Sabeet®showed better activity than placebo group subjects. Statistical analysis using Analysis of Co-Variance (ANCOVA) showed that every efficacy parameter, reached statistically significant difference ($p<0.05$). The change in the efficacy assessments was significant ($p<0.05$) between the two treatment groups (mean values) when their respective Day 0 and Day 14 were analyzed. There were no protocol deviations or violations in this study.

DISCUSSION

Beetroot is particularly rich in inorganic nitrate content (typically ranging from 110 to 3670 mg nitrate•kg−1) [37].

Nitrate may be reduced to its antecedent's nitrite and nitric oxide in vivo, particularly in environment such as in hypoxia and acidosis. Research investigating the physiological actions of nitrate has reported effects such as improvement of vascular compliance (Bahra et al., 2012), reduction of blood pressure (Larsen et al., 2007), and attenuation of oxidative stress (Carlström et al., 2011) following consumption. Given these properties, nitrate is commonly used as a pharmacological agent to treat a host of cardiovascular pathologies (Butler &Feelisch, 2008).

Lansley and colleagues [38] demonstrated that dietary NO_3^-, administered in the form of beetroot juice (500 ml/day for 6 days), decreases resting systolic blood pressure (SBP) and O_2 consumption during walking and running.

The BP-lowering effects of inorganic nitrate may attributed to increased generation of nitric oxide (NO) [39, 40], a

pleiotropic molecule involved in the vasodilation of large arteries and resistance vessels [39, 40].

Reduced NO bioavailability has been associated with impairment of endothelial function and increased risk of hypertension and cardiovascular diseases [41–44].

In addition to its therapeutic use, nitrate supplementation has recently been studied for its potential to enhance exercise performance. At present, the research of nitrate as an ergogenic aid for exercise performance is in its infancy.

Intense exercise, especially to exhaustion, has been shown to increase free radical concentrations in the muscles and liver by two to three times. Interestingly, several recent investigations have examined the potential antiradical properties of certain constituents of BRJ, namely betacyanins and betaxanthins, the main pigments of red beetroots [45].

In addition, Kanner et al reported that linoleate per oxidation by cytochrome c was inhibited by betanin from red beets [46].It was suggested that regular beetroot consumption may provide protection against certain oxidative stress-related disorders in humans, and therefore may serve as a useful strategy to enhance recovery from exercise and subsequent exercise performance.

In the present study, twenty healthy subjects who received Sabeet® for a period of 2 weeks, as one sachet per day before an exercise bout, reported a significant change in their exercise performance towards end of the study, than Placebo receiving subjects. Importantly, serum inflammatory biomarkers exhibited due to exercise bouts demonstrated a decreased trend in Sabeet® but no change in the Placebo group subjects. There were no study product related adverse events. With no abnormal laboratory values, changes in the vital signs, and with no statistical difference ($p > 0.05$) between both the treatment groups, Sabeet® as dietary supplement could be confirmed showing good efficacy for cardiovascular performance. Furthermore, Sabeet® was also found to be showing good benefits for exercise performance.

These additional benefits of Sabeet® can enhance cardiovascular and exercise performance. However exclusive clinical studies on larger population are recommended.

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