Phytochemical, cardiovascular effect, antioxidant, anti-inflammation and anti-tumor properties by beta vulgaris (beet) root juice

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Abstract

In the present work, we have investigated phytochemical content, cardiovascular effect, anti-oxidant, anti-inflammation, and anti-tumor by Beta Vulgaris (Beet) Root Extract Juice. It is highly abundant and is commonly known as “Cu Den” in Vietnam. Ayurvedic literature reveals that Beet Root extract has cardiovascular effect, antioxidant, anti-inflammation, and anti-tumor properties. Beta Vulgaris (Beet) root Juice Extract traditionally have been traditionally used as medicinal, nutritional and health benefits, besides its rich supply of mineral and vitamins, its antioxidant property help to prevent the formation of cancerous tumors and are therefore a powerful cancer-fighting agent. In this research articles, we discuss the biological fate of Beet Root extract juice with special emphasis on dietary inorganic nitrate in cardiovascular systems. Phytochemical analysis of Beet Root was detected by ICP-OES. Effect of Dietary Nitrate on Blood Pressure was measured Systolic Blood Pressure and Pulse Rate. The Effectiveness of Beet Root Extract on induction of cancer cell apoptosis and necrosis was measured by low and high N fertilization level. The antioxidant property of Beet Root extract was measured by using the growth medium as % of fermented juice of total weight in the wells. Betalain levels were measured by using Robust analytical HPLC-DAD-MS techniques.

Introduction

Beetroot (Beta Vulgaris) as a flavor agent, which includes silver beet, sugar beet, fodder beet and beet; it is a member of Chenopodiaceae family (Figure 1). Beetroot produces swollen root during growing season, and green tops. It is presently distributed over the whole world especially in South America included southern part of Mexico, India, Thailand and Vietnam; but originally from Germany. It usually grown for salad rather than for sugar production. It can be cooked, blanched, steamed or boiled. Beetroot is one of the richest dietary sources of dietary nitric oxide. It improves blood flow though the body including the brain, heart and muscles. It helps open up the cardiac vessels and allows more oxygen flow in; it also decrease the incidence of cardiovascular disease by lower both systolic and diastolic pressure mechanism.

In vivo, we have shown that ingestion of beetroot juice (dietary nitrate) results in increased plasma nitrite concentration. It substantially decreases blood pressure, inhibits platelet aggregation, and prevents endothelial dysfunction in all healthy patients. The study had a randomized, double blind, crossover design, the treatment periods were separated by a washout period of at least 10 days. The reduction in blood pressure continued to be observed until up to 24 hrs after the juice was consumed. Systolic blood pressure and pulse rate didn't change significantly after dietary nitrite supplement were given. However, the diastolic blood pressure was about 4mmHg lower.

To be the most important components of diet for good health is consuming the plant products. Vegetables and fruits have been shown to have nutritional and therapeutic effect. Phytochemical from Beetroot juice have been found to be responsible for such effects includes vitamins, mineral, and therapeutic effect. Phenolic compounds such as Flavonoids, phenolic acids, and phenolic amides, Betanin Isobetanin, Vulgaxanthin I &II. They all exhibit anti-ischemic effect, anti-inflammation effect, antioxidant, and anticancer effect.

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This work investigated some physicochemical properties of Beetroot juice. In Southern California, where we have similar tropical weather country like Asia, it is also the best place in US to grow Beetroot trees. In Southern California, USA with the help of Herbalife Nutritional Translational Research and Clinical Pharmacology, we evaluated the Beetroot juice. Beetroot was destalked, washed, sliced into thin piece, and grated into mash. Juice extracted of mash to water, and sugar syrup added. Finally, it is pasteurized at 85 degree Celsius, cooled at room temperature.

Then, this beetroot juice was given to healthy volunteer patient for measure blood pressure in New York, Ohio State and Herbalife Employee in Southern California, USA. They were also given to solid tumor cancer patients in New York, Ohio State, USA and HCM City, Vietnam for anti-tumor effect evaluation. Data have been measured and analyzed by statistics, lab analyst. These researches have been taken place places in New York, Ohio, Southern California, USA and HCM City, Vietnam from Jan 2016 to June 2018.

Materials and methods

Mineral component of beetroot juice

Oven drying method significantly increased the potassium value of the juice to 875mg compared to the fresh juice sample (230mg) with the least amount of potassium value and showed significant difference (p>0.05) in all the juice samples. The fresh juice had the highest iron content (21mg); followed by oven dried sample with 16mg of iron and sun dried 15mg of iron. The calcium content of the juice isn’t affected by the sun drying method. With drying method, Magnesium value within the juices was increased; Oven dried sample significantly increased to 102mg, sun dried sampled to 51mg compared to the fresh juice with Magnesium value of 34mg. The result showed that by the method of drying as depicted in Table 1, the nitrate content of juice sample was not influenced.

Dietary nitrate lowers BP

There were no differences in BP between the 2 groups during the hour before ingestion of beetroot juice or water. BP began to decrease from 1 hour after ingestion of juice compared to the water control (Figure 2). The peak difference in systolic BP was achieved at 2.5 hours after ingestion with a drop a 11+-2mm Hg (P<0.01), whereas the peak differences in diastolic BP and MAP were seen at 3 hours after ingestion it changes of 9+-2mm Hg and 8.2 +/-mmHg, respectively (both P<0.01), (Figure 2). At 2.4 hours, systolic BP was 4.5mm Hg lower with beetroot juice than water. However, systolic BP was significantly reduced by approximately 6mm Hg at 24 hours after beetroot juice ingestion compared to -1 hour. The mean heart rate was not significantly altered over 1 to 6 hours period after beetroot juice or water ingestion.

Mechanism of beetroot juice metabolism

After ingestion of beetroot juice plasma juice plasma nitrate concentration increased rapidly (within 30 minutes), peaking at 1.5 hours. Up to 25% of plasma nitrate is also excreted in the saliva [1,2], but the vast majority of absorbed inorganic nitrate is ultimately excreted in the urine. The result is the provision of substrate for the nitrate reductases expressed by bacteria that colonize the dorsal surface of the tongue, resulting in the reduction of nitrate to nitrite. This nitrite is swallowed and in the acidic environment of the stomach is then reduced to Nitric Oxide (NO) or reenters the circulation as nitrite (Figure 3). Nitrite mediates this decrease in BP, recent evidence demonstrates that nitrite is potent vasodilator in human [3], and it is likely that such vasodilator activity underlies the BP effects evidenced. This activity of nitrite has been attributed to its chemical reduction to the potent vasodilator Nitric Oxide (NO). That nitrite derived from dietary nitrate was a substrate for Nitric Oxide Synthase (NOS)-independent production of Nitric Oxide (NO) in the acidic conditions of the human GI tract [4,5].

| Mineral Components (mg) | Fresh Juice Sample | Sun dried Juice Sample | Oven Derived Juice sample |
|-------------------------|--------------------|------------------------|--------------------------|
| Nitrate                 | 0.04               | 0.04                   | 0.04                     |
| Potassium               | 230                | 327                    | 875                      |
| Iron                    | 20                 | 15                     | 16                       |
| Calcium                 | 3.1                | 3.6                    | 3.9                      |
| Magnesium              | 34                 | 51                     | 100                      |

Figure 1. Beetroot (Beta Vulgaris) as a flavor agent

Figure 2. The effect of beetroot juice on the change from baseline in (a) diastolic BP, (b) systolic BP, (c) heart rate, (d) effect of spitting vs. swallowing of saliva on the changes in systolic BP after beetroot juice. *P<0.05, **P<0.01, ***P<0.001

Figure 3. The metabolism of dietary nitrate, derived from consuming beetroot juice The metabolism of dietary nitrate, derived from consuming beetroot juice

Table 1. Mineral components of the beetroot juice

The metabolism of dietary nitrate, derived from consuming beetroot juice
Betalains are having high antioxidant and anti-inflammatory capabilities and variety in vivo human. It has a wonderful role for beetroot in clinical pathologies characterized by oxidative stress and chronic inflammation such as liver disease, arthritis and even cancer (Figure 4).

The betanin, the most abundant betalain found in beetroot (300-600 mg.kg-1), was the most effective inhibitor of lipid peroxidation. Betalains' high antioxidant activity appeared to stem from its exceptional electron donating capacity, and its abilities to defuse highly reactive radicals targeting at cell membranes [11]. Beetroot also contains several highly bioactive phenolics, such as caffic acid, pedicatechin and rutin which are also known to be excellent antioxidant [12,13]. Nitrite and other Nitric Oxide (NO), as well as beetroot have been shown to suppress radical formation and directly scavenge potentially damaging Reactive Oxygen and Nitrogen Species (RONS) (i.e. UV radiation, xenobiotics) such as hydrogen peroxide and superoxide suggesting nitrate may also exhibit antioxidant effects [14-16]. Beetroot juices inhibited in vivo formation in the 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is mainly found in ovary cancer, nasal, and paranasal sinus cancer. Interestingly, the antioxidant capacity of beetroot juice in DPPH was far greater than more well-known vegetable juices, such as carrot, tomato, and fruit juices such as pineapple and orange juice (Figure 5).

Beetroot extracts and Betalains have emerged as potent anti-inflammatory agents. It is interfering with pro-inflammatory signaling cascades. The most important of these is the Nuclear Factor-Kappa B (NF-kB) cascade, it directly activates and transcribes most gene targets that regulate and amplify the inflammatory response (i.e., chemokines, cytokines, phagocytic and apoptotic cell) [17]. As the result, NF-kB activity plays a central role in the inflammatory processes that manifest in chronic disease. Betalains have also been shown to markedly suppress cyclooxygenase-2 (COX-2) expression, which is an important precursor molecule for pro-inflammatory arachidonic acid metabolites known as prosta glandins [18,19]. Previous study [20] also found higher concentration of betalains compared to phenolic compounds. This raises the possibility question for pharmaceutical industry that betalains rich beetroot supplements in sufficient doses could exhibit anti-inflammatory effects to newly promised synthetic drugs (Figure 6).

Interestingly, therapeutic administration of Beetroot juice (betalin rich) alleviated inflammation and pain in osteoarthritis patients. After 10 days continuously drinking 500mL a day, the pro-inflammatory cytokines, tumor necrosis factor-alpha (TNF-alpha) and interleukin-6 (INL-6), had decreased from 40% to 9%, and from 29% to 20%, respectively.

**Vitamin C and phenolic compounds**

The vitamin C content was measured according to the America Standard. The method for measuring Vitamin C content consists of the oxidation ofL-ascorbicacid (ASC) to de hydroxylascorbic (DHA) acid in an acidic environment using the blue dye 2,6-dichlorophenylindophenol. Phenolic compounds were determined following the method described by Hallmann [21], using high-performance liquid chromatography (HPLC). The samples were then centrifuged at 3920 x g. 1mL extract was collected from the test tube and re-centrifuged at 22.8xg. 500mMl extract was transferred to HPLC vials and analyzed.

**Induction of cancer cell apoptosis and necrosis**

The impact of the fermented beetroot juice extract on ovary cancer cell line is showed on Table 2. The extracts of organic beetroot juices induced higher levels of late apoptosis and necrosis in Ovary Cancer cells in relation to the conventional juice extracts, and beetroots.
grown with a low level of nitrogen fertilization (LNF). The highest concentration of extract induced significantly greater early apoptosis in relation to the other concentrations. At the same time, it activated the lowest level of late apoptosis and necrosis in Ovary Cancer.

The study of apoptosis was conducted by marking with annexin V and propidine iodide, which enables the identification of cells in the early and late phases of apoptosis and necrosis. Application of annexin V joined with Fluorochrome can detect surface apoptosis and necrosis using a flow cytometer (FACSCibur). The culture was kept for 3 days on 24 well culture plate, 100 thousand cells per well (Table 3).

Thus, it demonstrated that extracts of beetroot juices made from ORG beetroots caused a stronger effect on late apoptosis and necrosis in these tumor cells than those from CONV one, it proved betacyanins hampered cancer processes.

Analysis of correlation between the content of antioxidants in beetroot juice and the response of Ovary and Nasal Carcinoma

A significant positive correlation between the content of Vitamin C in ORG juices and early apoptosis was found for a concentration of 0.025% extract. The positive correlation between betanin-3-O-glucoside content in ORG fermented juice extracts and late apoptosis was recorded in extract of 0.025% concentration. There is a negative correlation was noted between the betanin-3-O-glucoside content in CONV juice and late apoptosis of Ovary cell for the extract of 0.0125% concentration Table 2.

Table 2. The effect of organic (ORG) and conventional (CONV) growing systems using low (LNF) and high (HFN) N Fertilization level, concentrations of extracts of fermented beetroot juice effect on the early, late apoptosis, necrosis and living cells

| Factor               | Early Apoptosis | Late Apoptosis | Necrosis | Living Cells |
|----------------------|-----------------|----------------|----------|--------------|
| ORG LNF level        | 56.2±11.0a      | 265.2±94.0b    | 215±42.2b| 90.2±6.1a    |
| CONV LNF level       | 120.2±25.7b     | 109.2±58.4a    | 82.8±25.2a| 107.4±6.2b   |
| ORG HFN level        | 74.2±14.2a      | 256.2±115.4a   | 200.2±63.2a| 92.4±8.2a    |
| Extract (ORG+CONV) 0.025% | 62.2±15.1a    | 269.2±76.1b    | 175.2±361.2b| 95.1±3.1a    |
| Extract (ORG+CONV) 0.0125% | 79.2±13.2a     | 267.2±101.6b   | 206.2±60.1b| 90.2±6.2a    |

Table 3. The correlation relations between Vitamin C, Phenolic Acid vs. Ovary, Oral and Nasal Carcinoma Cancer cell

| Compounds       | Extract Concentration | Early Apoptosis | Late Apoptosis | Necrosis | Living Cells |
|-----------------|-----------------------|-----------------|----------------|----------|--------------|
| Organic Vitamin C | 0.025%               | +0.6            | NS             | NS       | NS           |
| Flavonoids      | 0.05%                 | NS              | NS             | +0.65    | NS           |
| Phenolic Acids  | 0.05%                 | NS              | +0.95          | +0.9     | -0.9         |
| Betanin-3-O-glucoside | 0.025%   | NS              | +0.7           | NS       | NS           |
| Betanin-3-O-Glucoside Conventional Juice | 0.0125% | NS              | -0.7           | NS       | -0.7         |
Conclusion

Beetroot (Beta Vulgaris) juice can mediate cardiologic effect, antioxidant, anti-inflammation and anti-tumor properties as well as chemotherapeutic agents. The result of different studies indicates its need for implementation for therapeutic advantage for the near future.

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