Inhibitory effect of beetroot (*Beta vulgaris)* juice extract, and its implication for cardiovascular disease.

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Abstract

Background: Beetroot (*Beta vulgaris*) is a bulbous purplish red root vegetable that can be used as a functional food because of its inherent phytochemicals, antioxidant properties and pharmacotherapeutic properties. Inhibition of arginase and lipase could improve cardiovascular health by increasing production of nitrous oxide and reducing obesity. This study was carried out to investigate the *in vitro* inhibitory effects of raw and heat-treated beetroot (*Beta vulgaris)* extracts on arginase and lipase enzymes. **Materials and Methods**: Arginase and lipase inhibitory activities of the raw and heat-treated beetroot extracts were elucidated in vitro, by adding the respective enzymes with dissolved freeze-dried beetroot extracts and the buffer - substrate complex. The ability of the beetroot extracts to inhibit the enzymes and as well as the respective IC_{50} values were determined by spectrophotometric methods. **Results:** There was a dose-dependent increase in the percentage inhibitions of arginase and lipase activities by raw and heat-treated beetroot. The heat-treated beetroot had the strongest inhibitory activity due to its lower half-maximal inhibition concentration (IC_{50}) values of 127.73 µg/mL and 373.11µg/mL for arginase and lipase respectively, when compared to the raw beetroot values of 133.68 µg/mL and 472.08 µg/mL respectively. **Conclusion**: The *in vitro* inhibitory effect of beetroot extract on arginase and lipase could give direction to carry out further studies on its ability to improve cardiovascular health. There is a need to investigate this potential *in vivo*.

Keywords: Beetroot juice, cardiovascular diseases, lipase, arginase.

Introduction

Medicinal plants are rich sources of broad-spectrum enzyme inhibitors (1). Flavonoids and polyphenols are in the large majority of noncompetitive, uncompetitive, or mixed type inhibitors (2). They could also act as chelators of minerals leading to disruption of enzyme conformation (3). Beetroot (*Beta vulgaris*) is a red-purplish and bulbous root vegetable that can be used as a functional food. Functional foods are nutrient-dense or fortified foods that promote optimal health and reduce the risk of disease. Some examples are beans, nuts, oats, cruciferous, vegetables and fermented dairy products. They contain inherent bioactive components that elicit health benefits particularly against chronic inflammation and oxidative stress (4,5). Betalain, betacyanin and betaxanthain have been identified as the pigments in beetroot that induce anti-inflammatory and antioxidant properties (6). The antioxidant content, antioxidant capacity and free radical-scavenging abilities of raw and heat-treated beetroot have been reported (7). Dietary nitrates have been evaluated in beetroots. The beneficial effect is attributed to *in vivo* reduction to Nitric oxide (NO), a multifarious messenger molecule with important vascular and metabolic functions (8). Inhibition of arginase increases the concentration of the substrate L-arginine and activation of Nitric oxide synthase (NOS). This leads to the beneficial production of

Nitric oxide (NO) in cells. Bioavailabilty of NO can be maintained by the administration of arginine (8). Nitric oxide is known to promote vasodilation and consequently reduce blood pressure in high resistant vessels (9). Stimulation of arginase decreases NO production due to enhanced NOS (Nitric oxide synthase) decoupling, thereby increasing the production of superoxide anions (10). Hence, inhibition of arginase stimulates the production of NO and reduces the level of superoxides (11). This leads to the dilation of blood vessels and consequently reduced blood pressure, control of hypertension and endothelial dysfunction (12). As studies have reported that incidence of vascular disorders such as atherosclerosis, diabetes, obesity and ischemia-reperfusion injury have been linked to reduced bioavailability of Nitric oxide (13,14).

Obesity is a metabolic disease which predisposes obese persons to certain diseases like cardiovascular disease, diabetes mellitus, cancer and osteoarthritis (15). Available treatment options for obesity include reduced intake of calories and increase in the consumption of fiber, regular physical exercise and medications ranging from lipase inhibitors and anorectics.

Lipase is a hydrolytic enzyme that breaks down triglycerides (fats). It is important in the digestion, transportation, and processing of dietary fat. The human pancreatic lipase plays a major role in the catabolism of dietary fats. It catalyses the conversion of triglyceride to monoglyceride and fatty acids. Recent approaches have shown that treatment of obesity treatment can be achieved by inhibiting dietary triglyceride absorption via pancreatic lipase inhibition (16,17). The effectiveness and therapeutic potential of enzyme inhibitors in the treatment of obesity and associated co-morbidities (like hypertension, fatty liver disease and type 2 diabetes) consolidate the need to search for new sources of natural inhibitors (18,19,20). Phytochemicals such as polyphenolics and flavonoids known to inhibit pancreatic lipase are reported to be abundant in beetroot (21,22). This study aimed to evaluate the *in vitro* inhibitory activities of raw and heat-treated Nigerian beetroot juice extracts on arginase and lipase activity.

Materials and methods

Materials

Sample Preparation

Fresh beetroots (3kg) purchased from the vegetable market at Airport road, Benin City, Edo state, Nigeria were washed with clean water to eliminate dirt, peeled to remove the skin and then grouped into two equal parts. The heat-treated beetroots in the first group were cut into 1-inch pieces, boiled at 100ºC for 30 minutes and strained using a wire mesh strainer while the raw beetroots in the second group were sliced and grated. The raw and heat-treated beetroot were blended separately with 2.25 Litres of distilled water until a smooth texture was obtained using an electronic blending machine (Binatone BLG-452 model, Japan). The already blended (raw and heat-treated beetroot were poured into respective airtight containers designated for each, dried using a vacuum freeze dryer (Armfield 2004 model, Germany) and stored at -18°C in a freezer until ready for use. The freeze-dried samples were composed of 10% beetroot juice containing 9808.0 mg GAE/100 ml polyphenols and 8334.0 mg QE/100 ml flavonoids (7).

Methods

The freeze-dried raw and heat-treated beetroot samples were prepared by dissolving 0.5g of the freeze-dried raw and heat-treated beetroot extract into 50mL of distilled water to get a concentrated solution of 10mg/mL. The freeze-dried pellets were homogenized in a glass mortar and pestle to get a uniform consistency solution and centrifuged at a speed 12,000 rpm for 20 minutes using a KX2400C, UK. The filtrate obtained was used for the assays.

In Vitro Arginase Inhibitory Activities of Beetroot Extract

A stock solution of 0.01g/mL of concentrated (raw and heat-treated) beetroot was used to prepare varied concentrations of 71-

286µg/mL of beetroot extracts via appropriate dilution of Tris buffer (Tris-HCl ＋MnCl; pH =9.5). Arginase enzyme (50µL) (Sigma-Aldrich, UK) and 50µL of L-arginine (substrate) were added to each test tube and incubated at 37ºC for 10 minutes. After incubation, 2.5mL of Ehrlich reagent was added to each test tubes and the entire mixture was allowed to stand for 20 minutes. Absorbance was read at 450nm using a spectrophotometer (Double Beam Uv-Vis spectrophotometer 2377, India). The reference sample (Control) was prepared using the same procedure except that the plant extract was replaced with 1mL of distilled water. The inhibitory activity of the extracts on arginase activity was calculated according to the formula shown below (23)

$$Inhibition(\%) = \frac{Absorbance_{450}(ref) - Absorbance_{450}(extract)}{Absorbance_{450}(ref.)} \times 100$$

The IC_{50} values (half maximal inhibitory concentration of the enzyme activity) were determined from plots of percentage inhibition versus inhibitor concentration and were calculated using linear regression analysis from mean inhibitory values. All tests were done in duplicate.

***In Vitro* Lipase Inhibitory Activities of Beetroot Extract**

Concentrated (raw and heat-treated) beetroot stock solution of 0.01g/mL was used to prepare varied concentrations of 180-710µg/mL of beetroot extract by appropriate dilution with buffer (glycine 0.5M adjusted to pH 1 with NaOH). Addition of 100µL pancreatic lipase enzyme (Sigma-Aldrich, UK) into each test tube was done before incubation at 37ºC for 5 minutes using a water bath. After incubation, 100µL of the substrate, p-nitrobenzylbutyrate (PNBB) (Sigma-Aldrich, USA) was added to the reacting test samples before incubating again at 37ºC for 5minutes to allow for a reaction between substrate and enzyme. The reaction was terminated by adding 200µL of isopropanol (Sigma-Aldrich, USA) to the respective test tubes. Absorbance was read at 410nm using a Uv-visible spectrophotometer (Double Beam Uv-Vis spectrophotometer 2377, India) The reference sample (Control) was prepared using the same procedure except that the plant extract was replaced with 1mL of distilled water. The inhibitory activity of the extracts on lipase activity was expressed as percentage inhibition, which was calculated according to the formula shown below (23)

$$Inhibition(\%) = \frac{Absorbance_{410}(ref.) - Absorbance_{410}(extract)}{Absorbance_{410}(ref.)} \times 100$$

The IC_{50} values (inhibition concentration at which 50% inhibition of the enzyme activity occurred) were determined from plots of percentage inhibition versus inhibition concentration and were calculated using linear regression analysis from mean inhibitory values. All tests were done in duplicate.

Statistical analysis

Results were expressed as mean ± SEM of duplicate determinations. The data were analyzed by one-way analysis of variance, followed by Duncan test to determine the level of significance which was expressed at 5% confidence interval ($p \leq 0.05$).

Results

The Percentage inhibition and IC_{50} of raw and heat-treated beetroot on arginase activity is presented in Table 1, Figure 1. There was a dose-dependent increase in percentage inhibition of arginase on the raw and heat-treated beetroot juices. The results showed a significant difference *at* ($p \leq 0.05$) when the dose was doubled for the raw and heat-treated beetroot. The heat-treated beetroot had a lower IC_{50} value than the raw beetroot. The inhibitory effect on lipase by beetroot is represented in Table 2, figure 2. A dose-dependent increase in percentage inhibition was also revealed. There was a significant difference *at* ($p \leq 0.05$) when the dose was doubled for the raw beetroot and the heat-treated beetroot. This was also reflected in the IC_{50} value. The heat-treated beetroot had a lower IC_{50} value than the raw beetroot.

Table 1. Percentage Inhibition and IC_{50} Values of Raw and Heat-Treated Beetroot on Arginase Activity

Concentration (µg/mL)	%Inhibition of raw Beetroot	IC_{50} (µg/mL) of raw beetroot	%Inhibition of heat-treated beetroot	IC_{50} (µg/mL) of heat-treated beetroot
71	35.675±4.87[a]		31.170±4.33[a]	
143	54.955±1.09[a]	133.68	58.735±5.61[ab]	127.73
214	64.145±6.31[ab]		77.655±10.19[b]	
286	84.24±1.90[b]		75.490±4.07[b]	

Data are expressed as mean ± SEM (Standard Error of Mean) of duplicate determination. Values with same superscript are not significantly different while those with different superscripts are significantly different at ($p \leq 0.05$)

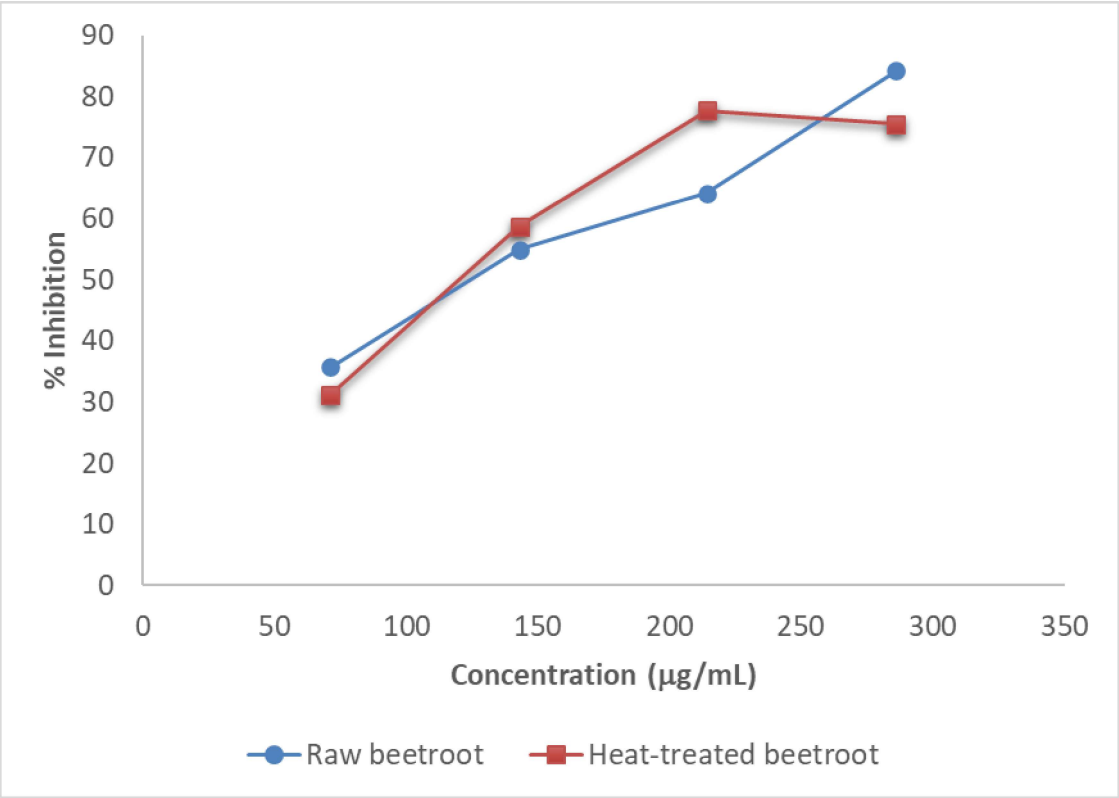

Figure 1: Determination of IC_{50} values of arginase by raw and heat-treated beetroot extract.

Table 2. Percentage Inhibition and IC_{50} of Raw and Heat-Treated Beetroot on Lipase Activity

Concentration (µg/mL)	%Inhibition of raw Beetroot	IC_{50} (µg/mL) of raw beetroot	%Inhibition of heat-treated beetroot	IC_{50} (µg/mL) of heat-treated beetroot
180	19.725±2.30[a]	472.08	25.685±2.59[a]	
360	36.235±1.38[b]		55.040±3.89[a]	373.11
540	62.39±3.67[c]		61.925±.3.25[ab]	
710	71.555±1.84[cd]		91.745±9.08[c]	

Data are expressed as mean ± SEM (Standard Error of Mean) of duplicate determination. Values with same superscript are not significantly different while those with different superscripts are significantly different at ($p \leq 0.05$)

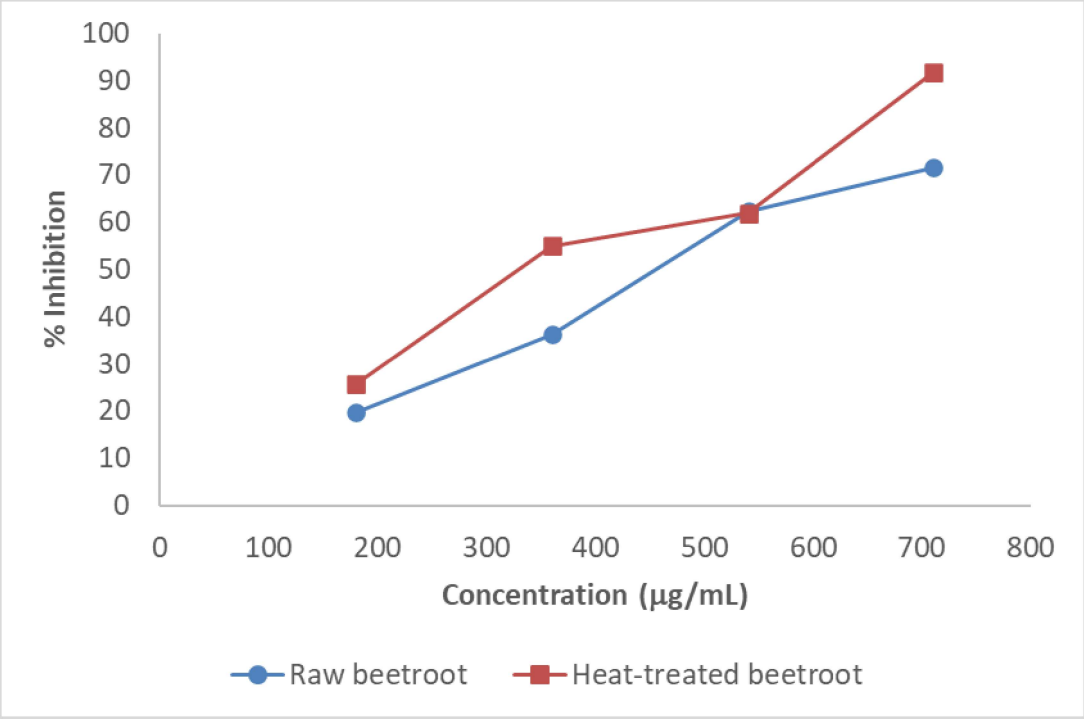

Figure 2: Determination of IC_{50} values of lipase by raw and heat-treated beetroot extract.

Discussion

The efficacy of plant-based diets in the management of obesity, type 2 diabetes and coronary heart disease (CHD) is due to the presence of phytochemicals, anthocyanins and polyphenols that can scavenge free radicals (24). Medicinal plants are used as natural alternatives in treatments of various ailments due to its cost-effectiveness and low incidence of adverse effects when compared to the conventional synthetic compounds (25).

Beetroot juice extract inhibit arginase and lipase in a dose-dependent increase in the percentage inhibitions by raw and heat-treated beetroots. The result of this study showed that the heat-treated beetroot had the strongest inhibitory activity on arginase and lipase as attested by its lower half-maximal inhibition concentration (IC_{50}) values of 127.73 µg/mL and 373.11µg/mL respectively when compared to the raw beetroot.

Phytochemicals such as ascorbic acid, carotenoids, phenolic acids and flavonoids

have been reported to be present in generous amounts in beetroot (7,22,26). Previous study showed that flavonoids alter the conformation of arginase due to its non-competitive inhibition (27). Quercetin is able to chelate manganese at the active site of the enzyme as a mechanism for the inhibition of arginase (28). This would most probably lead to increase in nitric oxide responsible for improved endothelial function and cardiovascular health (29,30). Polyphenols generally act by binding to the polyvalent sites of lipase (31) to inhibit it and reduce body weight, fat, and plasma free fatty acid level. Therefore, polyphenols are referred to as the major phytochemical that inhibit pancreatic lipase (31).

The inhibition of arginase by raw and heat-treated beetroot could be responsible for the hypotensive effect of beetroot (32). A decrease in the activity of arginase will result in the bioavailability of L-arginine. Arginine will activate the production of Nitric oxide via the catalytic activity of endothelial nitric oxide synthase (eNOS) (33). Nitrous oxide in endothelial cells diffuses into the vascular smooth muscles to dilate the blood vessels and this would play a role in the lowering of blood pressure. The presence of vitamin C in beetroot inhibits the dissolution of nitric oxide in blood vessels (34). The antioxidant activity of beetroot juice also stimulates the nitric oxide synthase and promotes Nitric oxide production in the blood vessels. This mediates the relaxation of vascular smooth muscle and prevention of cardiovascular disease.

Conclusion

This study shows that the raw and heat-treated beetroot juice extracts inhibits lipase and arginase enzymes *in vitro*. There is a need to carry out further study to ascertain the *in vivo* inhibitory properties.

Conflict of Interest

The authors declare no conflicts of interest.

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