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Beetroot (*Beta vulgaris)* Juice Inhibits Key Carbohydrate Metabolising Enzymes Associated With Type II Diabetes

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ABSTRACT

Beetroot (*Beta vulgaris*) is a root tuber belonging to the Amaranthaceae family, which has recently become popular in Nigeria. The reddish purplish tuber and green leaves are usually eaten by boiling, roasting and the raw tuber can be made into juice. This study was carried out to investigate the in vitro ability of aqueous freeze-dried beetroot juice to inhibit α-amylase and α-glucosidase linked to type 2 diabetes (T2D) by spectrophotometric methods. The percentage inhibition and its half maximal inhibitory concentration (IC_{50}) of α-amylase and α-glucosidase by beetroot juice with starch revealed that beetroot juice inhibited α-amylase (26%-73%; IC_{50} = 1.78 ± 0.13 mg/mL) and α-glucosidase (53%-85%; IC_{50} = 0.77 ± 0.14 mg/mL) in a dose dependent manner. This was compared with the positive control acarbose which inhibited α-amylase (IC_{50} = 0.18 ± 0.02 mg/mL) and α-glucosidase (IC_{50} = 0.22 ± 0.01 mg/mL). Beetroot juice may possess hypoglycemic effects and through these inhibitory mechanisms, it could be used as an adjuvant in the management of hyperglycemia and diabetes.

Keywords: Beetroot juice, carbohydrate hydrolyzing enzymes, α-amylase, α-glucosidase.

INTRODUCTION

Beet (*Beta vulgaris*) is a root vegetable plant which belongs to the Amaranthaceae family. The plant is commonly grown in Jos, Northern Nigeria. Though not an indigenous plant, it is however gradually being introduced as a component of salad and as constituents of some fruit drinks. The bulbous root tuber of beetroots can be peeled and boiled, or the raw bulb processed into a pink-purplish juice. Beetroot juice is not usually consumed as other fruit and vegetable juices like tomatoes, carrot, apple, mango (Thakur and Das Gupta, 2006). The leaves and roots of beetroot are high in Vitamin A and C (Lintas, 1992) and essential minerals (Olumese and Oboh, 2016a). The plant is rich in protein and fibre (Ansari *et al.,* 2017). Beetroot has a medium glycemic index of 64 (Wootton-Beard *et al.,* 2014).

Beetroot is rich in bioactive compounds, phytochemicals and pharmacotherapeutic agents (Chawla *et al.,* 2015; Odoh and Okoro, 2013) and antioxidants containing polyphenols and flavonoids that are effective free radical scavengers (Georgiev *et al.,* 2010; Olumese and Oboh, 2016b). It can therefore be used as a functional food because it contains pigments called betalains, a class of betalamic acid composed of betacyanins and betaxanthins that are rich in polyphenols and anthocyanins (Pitalua *et al.,* 2010). These bioactive compounds have been reported to reduce both postprandial hyperglycaemia and prevent hyperinsulinaemia by reducing the digestion, absorption and transport of glucose (Bahadoran *et al.,* 2013; Dragan *et al.,* 2015) with a corresponding stimulation of insulin release (Vinayagam *et al.,* 2016). Beetroot juice have also been shown to lower blood glucose level with a concomitant decrease in serum insulin and C-peptide levels and a corresponding elevation of cortisol in healthy subjects (Olumese and Oboh, 2016c). This study was carried out to determine the ability of the polyphenolic rich Beetroot juice (BRJ) to inhibit the key enzymes α-amylase and α-glucosidase linked to type 2 diabetes.

MATERIALS AND METHODS

Sample Collection and Treatment

Beetroot (*Beta vulgaris*) was obtained from a vegetable market, Airport road, Benin City, Nigeria. The plant was identified by a

Taxonomist at the Department of Plant Biology and Biotechnology at the University of Benin with a voucher number UBH_B 374 deposited in the Herbarium. The dark red bulbous roots of the beetroots were rinsed with distilled water, 10 kg of beetroot was peeled and blended with three litres (3L) of distilled water in a Moulinex Blender LM 2411, Wanette, Oklahoma, USA. The juice was filtered using a muslin cloth, allowed to settle for 20-30 minutes. The filtrate obtained was then freeze dried [Armfield vacuum freeze dryer Model FT 33, England]. The freeze-dried sample was stored at 0 $^{\circ}$C until ready for use.

Chemicals and Reagents

The enzyme porcine α-amylase [EC 3.2.1] and α-glucosidase [EC 3.2.1.20] were obtained from Sigma-Aldrich chemie GmbH (Steinheim, Germany) and Sigma-Aldrich (St. Louis, MO, USA).

Sample Preparation

One gramme (1 g) of the freeze-dried extract of the beetroot (*Beta vulgaris*) was dissolved in 25 ml of distilled water in a beaker and left to stand for 24 hours in a covered container. A 1 % starch solution was prepared by dissolving 1g soluble starch in an initial volume of 80 ml of 0.02 M sodium phosphate buffer, pH 6.9. The solution was allowed to boil while stirring. Thereafter, the temperature was maintained below boiling point for 15 minutes to solubilise the starch solution and allowed to cool to room temperature. The total volume was then made up to 100 ml by adding distilled water.

Enzyme Assay

Alpha-glucosidase inhibition assay

α-glucosidase activity was determined by the modified method described by Oboh *et al.* (2012). Appropriate dilutions of the extracts (0, 50, 100, 150, 200 µL) of the beetroot extract representing 0.8 mg/ml, 1.6 mg/ml, 2.4 mg/ml and 3.2 mg/ml were mixed with 100 µL of α-glucosidase (EC 3.2.1.20) solution (1.0 U/mL) in 0.1 mM phosphate buffer (pH 6.9) respectively and incubated at 25°C for 10 minutes. Then, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 mM phosphate buffer (pH 6.9) was added. The mixture was incubated at 25°C for 5 minutes, absorbance was read at 405nm. The α-glucosidase inhibitory activity was expressed as percentage inhibition calculated using the formula:

% of inhibition = (Absorbance of control - Absorbance of sample)/Absorbance of control x 100

Alpha-amylase inhibition assay

α-amylase activity was determined by the method described by Worthington (1993a). Appropriate dilutions of the beetroot extracts (0, 50, 100, 150, 200 µL) representing 0.8 mg/ml, 1.6 mg/ml, 2.4 mg/ml and 3.2 mg/ml of the beetroot extracts were mixed with 500 µL of 0.02 M Sodium Phosphate Buffer (pH 6.9 with 0.006 M NaCl) containing porcine pancreatic amylase [EC 3.2.1.1] [0.5 mg/ml] and then incubated at 25°C for 10 minutes. Then 500 µl of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M NaCl) was added to the reaction mixture in the test tubes respectively. The reaction mixture was incubated at 25^{o}C for 10 minutes and stopped with 1.0 ml of dinitrosalicylic acid (DNSA) colour reagent, followed by incubation in boiling water (100 °C) for 5 minutes and allowed to cool at room temperature. The reaction mixture was diluted by adding 2.5 ml of distilled water.

The control included all other reagents and the enzyme except for the test sample. Acarbose was used as a positive control. The mixtures were incubated at 25°C for 5 minutes, before reading the absorbance at 540 nm in the spectrophotometer.

The percentage of amylase inhibition by the extract was subsequently calculated as follows:
% of inhibition = (Absorbance of control - Absorbance of sample)/Absorbance of control x 100

Data analysis

The graph of % enzyme inhibition was plotted against extract concentration. IC_{50} is the concentration needed to inhibit 50 % of the enzyme activity (α-glucosidase and α-amylase) under the described assay conditions. This was calculated using graph pad prism version 6.1 for windows.

RESULTS

Enzyme inhibition assay

This study showed that beetroot juice inhibited the activities of α-glucosidase and α-amylase in a dose-dependent manner. Figure 1 shows the different doses of beetroot juice 0.8 mg/ml, 1.6 mg/ml, 2.4mg/ml and 3.2mg/ml gave a percentage inhibition of α-glucosidase of 53.5 %, 61.7 %, 73 %, 85.25 % respectively. The half maximal inhibitory concentration (IC_{50}) that is capable of inhibiting 50 % of the enzyme activity was found to be 0.77 mg/ml of the extract.

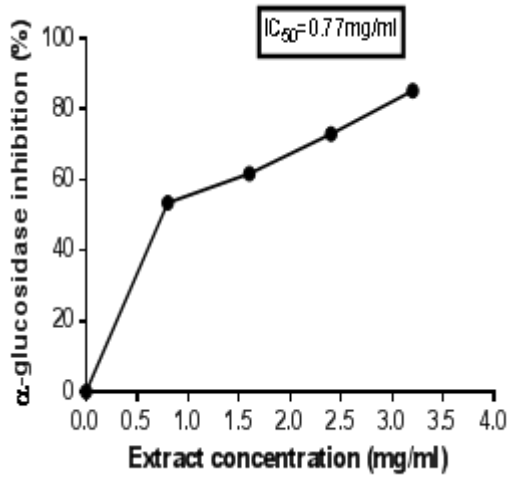

Figure 1: Beetroot juice inhibitory activity on α-glucosidase invitro

α-amylase inhibition studies (Figure 2) revealed that the different concentration of beetroot extract 0.8 mg/ml, 1.6 mg/ml, 2.4 mg/ml and 3.2 mg/ml gave a percentage inhibition of 26.22 %, 41.60 %, 59.44 % and 73.42 % respectively. The IC_{50} for inhibition of α-amylase was determined to be 1.78 mg/ml.

Acarbose (0.08 mg/ml) which was used as a positive control had IC_{50} 0.22mg/mL and 0.18 mg/mL respectively for α-glucosidase and α-amylase (Figures 3 and 4)The in vitro inhibitory effect of acarbose on α-glucosidase and α-amylase were observed to be higher when compared to beetroot aqueous extract

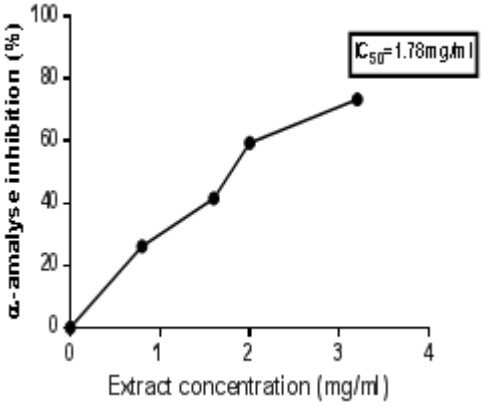

Figure 2: Beetroot juice inhibitory activity on α-amylase in vitro

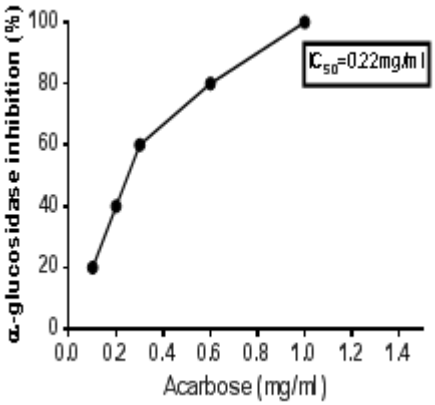

Figure 3: Acarbose inhibitory activity on α-glucosidase in vitro

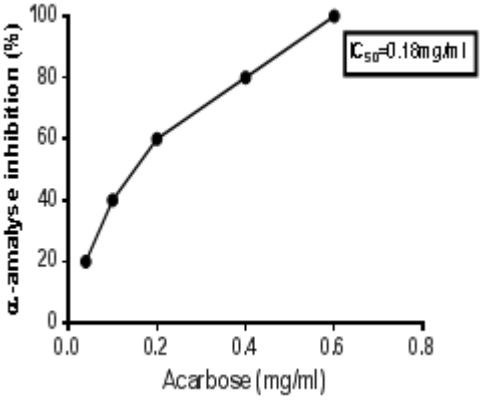

Figure 4: Acarbose inhibitory activity on α-amylase in vitro

DISCUSSION

Research have shown that substances that inhibit key enzymes such as α-amylase and α-glucosidase in the digestion of carbohydrates (starch blockers) are important, as they can be used in the management of type 2 diabetes (McCue *et al.,* 2005; Adefegha and Oboh, 2013). The inhibition of these enzymes by these starch blockers slow down the digestion of carbohydrates and consequently slow down the absorption of glucose from the gut into the blood stream (Kwon *et al.,* 2006; Oboh *et al.,* 2010). This delays a sharp rise in postprandial blood glucose following a carbohydrate meal. Many drugs used in the management T2D such as acarbose act through this mechanism. Bioactive compounds from natural sources with the potential to inhibit these key enzymes involved in the digestion of carbohydrates may be relatively cost effective and probably less toxic when compared to synthetic starch blockers (Adefegha and Oboh, 2013). Moreover, these bioactive compounds from food sources may act synergistically in the management and possible prevention of type 2 diabetes.

This study showed beetroot juice inhibited α-amylase within the range of 26%-73% in a dose dependent manner, with an IC_{50} of 1.78 mg/mL. Similarly, α-glucosidase was inhibited in the range 53%-85%, IC_{50} = 0.77mg/mL. Studies by Nwanna *et al.* (2013) using methanolic extracts of eggplant *S. marcocarpon* and *S. melongena* were seen to inhibit α-amylase and α-glucosidase in a dose dependent manner. However, this current study revealed that the inhibition of these enzymes by beetroot aqueous extract was slightly lower than that of acarbose (0.08mg/ml) which was used as the positive control and inhibited α-amylase with IC_{50} = 0.18 mg/mL and α-glucosidase with IC_{50} = 0.22mg/mL. The inhibition by beetroot juice is stronger for α-glucosidase and the IC_{50} for this inhibition is relatively close to that of acarbose. This agrees with researches done by Adefegha and Oboh (2013), Thilagam *et al.* (2013) in which plant extracts showed a stronger inhibition of α-glucosidase when compared to the inhibition of α-amylase.

Beetroots have been shown to be rich in polyphenol content and other bioactives such as flavonoids, betalains and ascorbic acid (Murthy and Manchali, 2013; Olumese and Oboh, 2016b). Several studies have correlated α-amylase and α-glucosidase inhibition by plant extract to their polyphenol content (McCue *et al.,* 2004; Husni *et al.,* 2014; Lim and Loh, 2016; Wang *et al.,* 2016). Reports on the mechanism by which this inhibition is achieved is varied. McCue *et al.* (2004) suggested that amylase inhibition may be due to the disruption of disulphide bridges on the surface of the enzyme by polyphenols, however the inhibition of α-glucosidase may be through other mechanisms. Other studies report that the mechanisms of these inhibitions may be competitive, non-competitive or mixed competition (Kazeem *et al.,* 2013; Wang *et al.,* 2016). Nevertheless, the mechanism through which these plant bioactives inhibit these enzymes may be through a synergy of mechanisms. The inhibition of α-amylase and α-glucosidase by aqueous beetroot juice in this study might follow the same mechanisms by polyphenols, moreover, juicing causes more polyphenol to be released through rupturing of the cell membrane, thus making them available (Olumese and Oboh, 2016b). Studies suggest that aqueous extract are relatively stronger inhibitors of these enzymes than other extracts (Kazeem *et al.,* 2013).

CONCLUSION

In this study, it was found that beetroot aqueous extract inhibits the carbohydrate hydrolyzing enzymes α-amylase and α-glucosidase *in vitro* in a dose dependent manner. This inhibition could promote gradual release of glucose into blood postprandial which could be effective in the management of type 2 diabetes.

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CONFLICTS OF INTEREST AND OTHER DISCLOSURES

The authors have no conflict of interest related to this study.

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