Effects of Daily Intake of Beetroot Juice on Blood Glucose and Hormones in Young Healthy Subjects

Article *in* Nigerian quarterly journal of hospital medicine · November 2016

CITATIONS
3

READS
1,926

2 authors:

Fidelis Ehidiamen Olumese
University of Benin
12 PUBLICATIONS **53** CITATIONS
SEE PROFILE

Henrietta Oboh
University of Benin
35 PUBLICATIONS **263** CITATIONS
SEE PROFILE

Effects of Daily Intake of Beetroot Juice on Blood Glucose and Hormones in Young Healthy Subjects

Olumese FE and Oboh HA

Department of Medical Biochemistry
College of Medical Sciences, University of Benin, Benin City, Nigeria

*Address correspondence to **Olumese FE**, E-mail: fidelisolumese@yahoo.com

ABSTRACT

Background: Hyperglycaemia occurs due to alteration in carbohydrate, fat or protein metabolism and hormones may be implicated. Millions of people now use medicinal plants to treat this condition.

Objectives: To determine the potential hypoglycaemic properties of Beetroot (*Beta vulgaris*).

Methods: Thirty subjects: (Eighteen males and twelve females) aged 19-29 years, BMI ⩽ 25 received 10% Beetroot juice daily. The juice was administered after overnight fast (Phase I), daily for six weeks (Phase II) and two weeks wash out (Phase III). Serum glucose, cortisol, insulin and C-peptide were evaluated.

Results: While the plasma glucose was significantly ($p<0.05$) reduced, serum C-peptide and insulin were down regulated with a concomitant increase in cortisol in the phase II compared to other phases.

Conclusion: Daily consumption of 10% Beetroot juice reduced blood glucose which may be beneficial to diabetics and this is supported by the levels of Insulin, cortisol and C-peptide.

Keywords: Beetroot , C-peptide, Glycaemic response, Insulin, Cortisol,

INTRODUCTION

Diabetes mellitus is an endocrine disorder which is characterized with chronic hyperglycaemia. It is a global disease affecting over 382 million people in the world ((International Diabetes Federation)[1]. Diabetes is caused by the deficiency of insulin which may be as a result of the destruction of pancreatic β - cells or resistance to insulin action[2]. The disease features *in vivo* as impairment in insulin stimulated glucose uptake in peripheral tissues[3] and the inability of target tissues, liver, muscle and adipose tissue to respond to insulin[4]. The frequency of the disease is increasing everyday due to aging, poor physical activity, obesity and urbanization.

Uncontrolled diabetes can lead to microvascular complications affecting the eyes, kidneys, nerves and cardiovascular health[5]. Diabetes is also associated with increased risk of cancer, arthritis, chronic liver disease, heart failure and amputations. The effective treatment of the disease is either by oral hypoglycaemic drugs or by intramuscular application of insulin[6,7]. The high cost of the drugs and its attendant burden on health care has made the management of diabetes difficult. There has been an increasing desire for natural, safer and effective alternative to these drugs. Tremendous research is on going to provide effective natural products with antidiabetic properties[8,9].

Red beetroots (*Beta vulgaris rubra*) has been reported to be a rich source of phytochemicals and health promoting bioactive compounds like polyphenols, flavonoids and anthocyanins[10]. Beetroot is also one of the few vegetables that contain a group of highly bioactive pigments that are red-violet in colour known as betalains, betacyanin or betaxanthin[11].

Beetroot is cultivated mainly in Jos, Northern Nigeria. It is not originally a tropical root crop and usually not incorporated into the Nigerian-like diet. Recently, the consumption of the bulbous root is gaining attention in most Nigerian homes. Recent literature has reported beetroot to possess antihypertensive, cardioprotective and hepatoprotective properties[12], inhibition of tumor cells and the process of apoptosis[13] . Beetroot is also being considered as a promising therapeutic treatment in a range of clinical pathologies associated with oxidative stress and inflammation[14].

There is however paucity of literature about the hypoglycaemic effect of beetroots. This study attempted to show the possible effects of Beetroot juice on blood glucose, cortisol, C-peptide and insulin concentration in the serum of healthy subjects.

SUBJECTS AND METHODS

Subjects

The subjects were recruited from different Faculties at the University of Benin. The study group consisted of 30 volunteers: 18males and 12 females, aged 19-29years old. The inclusion criteria was a normal glucose tolerance (FBS ⩽ 5.6 mmol/ L) and BMI less than 25 kg/m^2 They were not on any medication known to affect carbohydrate metabolism. An exclusion criteria was a blood glucose level above 6.5 mmol/L. The study protocol was carefully explained to the subjects before they signed a written informed consent. The College of Medical Sciences ethics committee approved the study (CMS/REC/2016/002).

Preparation of Experimental Juice.
Beetroot samples were purchased from the vegetable market on Airport Road, Benin City, Edo State, Nigeria. The Beetroot was washed, peeled and grated. The juice extract was prepared by blending the grated beetroot using an electronic blender (Saisho S-23,China) with distilled water to obtain a solution. The solution was allowed to pass through a mesh sieve, the residue was discarded and the filtrate used as the juice extract. A 10% solution was made daily. The supernatant was stored in a refrigerator at 0°C until utilized.

BIOCHEMICAL ANALYSES

Determination of Total Phenol Content
The total phenol content was determined according to the method of Singleton *et al.*[15]. The aqueous extract (100 µl) was oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance measured at 765 nm using JENWAY UV-Visible spectrophotometer. The total phenol content was calculated as gallic acid (10 mg/100ml) equivalent.

Determination of Total Flavonoid Content
The total flavonoid content was determined using a slightly modified method reported by Meda *et al.*[16]. The aqueous extract (100 µl) was mixed with 0.5 ml methanol, 50 µl 10% $AlCl_3$, 50µl 1M Potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was measured at 415 nm in the JENWAY UV-Visible spectrophotometer. The total flavonoid content was calculated using quercetin (10 mg/100ml) as standard.

Experimental Design
Blood samples were collected from subjects on Day 0 (Phase I: Control). The subjects were given a 10% beetroot juice (BRJ)[19] solution daily for six weeks and blood samples collected (Phase II: Intervention).Thereafter, a two week wash out period (Phase III: wash out period, no BRJ administered). Blood samples were collected from the subjects on the cubital fossa veins in an aseptic procedure between 8-9 am after an overnight fast of 12hr at the end of each phase. The samples were collected in fluoride oxalate containers for glucose determination, plain bottles (no anticoagulant) for insulin, cortisol, C-peptide and centrifuged for 10 min at 4000 rpm using an 80-2 centrifugal machine Trade mark Jactermac Germany. After centrifugation the plasma was immediately used for glucose determination, while serum were collected in plain bottles and stored at -20 °C for analysis.

Measurement of Serum Glucose .
Glucose was determined by the glucose oxidase method of Barham and Trinder[17]. A Randox kit N9CAT.NO.GL364 was used. The procedure for the assay was as directed by the manufacturer. and GENESYS 10 Spectrophotometer at 500 nm was used to read the absorbance. Analysis was done in duplicates.

Measurement of Serum Cortisol
Cortisol was determined by the ELISA kit catalog No. CO103S by Calbiotech. Cortisol-enzyme conjugate solution and wash buffer method using the cortisol ELISA KIT CAT NO with an ELISA Reader at 420 nm[18]. Prior to assay all the reagents were allowed to stand at room temperature and mixed gently. To the coated strips were pipetted 25 µl of Cortisol standards, control and patient's sera, followed by 200 µl of Cortisol Enzyme Conjugate. The wells were thoroughly mixed for 10 sec and incubated for 60 min at room temperature (18-26 °C). The wells were washed three times with 300 µl of wash buffer, blotted on absorbent paper towels. To the wells were added 100 µl of TMB (3,3,'5,5'-Tetramethylbenzidine) substrate and incubated for 15 minutes at room temperature (18-26^0 C) which was accompanied by the addition of 50 µl of stop solution to all the wells. The plate was shaken gently to mix the solution and the absorbance read on ELISA Reader at 450 nm within 20 min after adding the stop solution.

Measurement of Serum C-peptide
C-peptide ELISA kit catalog No. CP179S by calbiotech was used for the determination of human C- peptide levels in serum. Prior to assay, all the reagents, serum, references and controls were brought to room temperature (18-26 ^{0}C). To the assigned wells were pipetted 50 µl of the appropriate standard, control or specimen and 100 µl Enzyme Conjugate. The plate was gently mixed for 15-20 sec and incubated at room temperature for 60 min. The liquid in the wells were removed and washed three times with 300 µl of wash buffer and blotted on absorbent paper towels. This was followed by the addition of 100 µl of TMB substrate to all the wells and incubated for 15 min at room temperature (18-26 ^{0}C). Thereafter, 50 µl of stop solution was added to each well and gently mixed for 15-20 sec. ELISA Reader was used to read absorbance at 450 nm within 15 min after adding the stop solution

Measurement of Serum Insulin
The Calbiotech insulin ELISA kit catalog No.ISI30D was used for the quantitative measurement of insulin in serum. Prior to assay all the reagents were allowed to stand at room temperature. To the coated strips 25 µl of Insulin standards, control and patient's sera were pipetted into appropriate wells followed by the addition of 100 µl of working Insulin Enzyme Conjugate to all wells, mixed for 10 sec and incubated for 60 min at room temperature (18-26^0 C). The liquid was removed from all wells, washed three times with 300 µl of wash buffer. The wells were blotted on absorbent paper towels, 100 µl of TMB substrate was added to each well and incubated for 15 min at room temperature. A 50 µl of stop solution was added to all the wells and shaken gently to mix the solution. The absorbance was read with an ELISA Reader at 450 nm within 15 min after adding the stop solution.

Statistical Analysis
The statistical analysis of the data obtained was with Statistical Package for Social Sciences (SPSS 22.0) package program for windows. Kolmogorov-Smirnov test was used to determine the data distribution and the difference between the arithmetic means was tested. The P value calculated with Kolmogorov-Smirnov test being smaller than $P < 0.05$ shows that there is significance.

RESULTS
this study shows that the 10% Beetroot administered to the subjects for this study contained 9808.0 mgGAE/100 ml of polyphenols and 8334.0 mgQE/100 ml of flavonoids. Table 1 and Figures 1-4 represents the results obtained before, during and after the consumption of Beetroot juice. The changes observed were mainly in the intervention or Phase II groups. Administration of Beetroot juice for six weeks led to significantly ($p< 0.05$) reduced blood glucose levels from 76.1 mg/dL to 49.8 mg/dL (Figure 1). This represents a

34.5% reduction. There were no significant differences between the blood glucose levels in Phase I (Control group) and phase III (washout period). Cortisol levels were significantly ($p<0.05$) increased from 86.85 ng/ml to 104.61 ng/ml in the phase II during the administration of Beetroot juice (Figure 3). There was a corresponding significant $p<0.05$ decrease in the values of insulin obtained during the intervention period from 9.42 µ IU/ml to 1.16 µIU/ml (Figure 2). C-peptide values were significantly lowered from 0.79 ng/ml to 0.41 ng/ml in the Phase II (Intervention). This value increased thereafter to 1.52ng/ml at the post intervention or wash out phase (Figure 4). Statistically analysis revealed that the C-peptide values were not significantly ($p > 0.05$) different between Phase I and Phase II.

Table 1. Effect of Beetroot Juice on serum Glucose C-peptide, Insulin and Cortisol in Healthy Subjects

	Control (Phase I)	Intervention (Phase II)	Post Intervention (Phase III)
Glucose (mg/dL)	76.1 ± 14.2^{a}	49.8 ± 11.3^{b}	75.7 ± 11.1^{a}
Insulin (µIU/ml)	9.42 ± 4.60^{a}	1.16 ± 0.79^{b}	6.64 ± 2.46^{a}
Cortisol (ng/ml)	86.85 ± 12.2^{a}	104.61 ± 32.00^{b}	93.05 ± 14.8^{a}
C-peptide (ng/ml)	0.79 ± 0.68^{ab}	0.41 ± 0.39^{a}	1.52 ± 0.83^{b}

Serum glucose, C-peptide, Insulin and Cortisol concentrations were expressed as mean ± SEM. Values with different superscript in a row are significantly different at $P<0.05$.

There was a significant decrease ($P<0.05$) in the serum concentration of glucose and insulin with a corresponding significant increase ($P<0.05$) in the cortisol in intervention phase when compared to the normal control. While, the C-peptide in the intervention phase was significantly reduced ($P<0.05$) when compared to the post intervention phase.

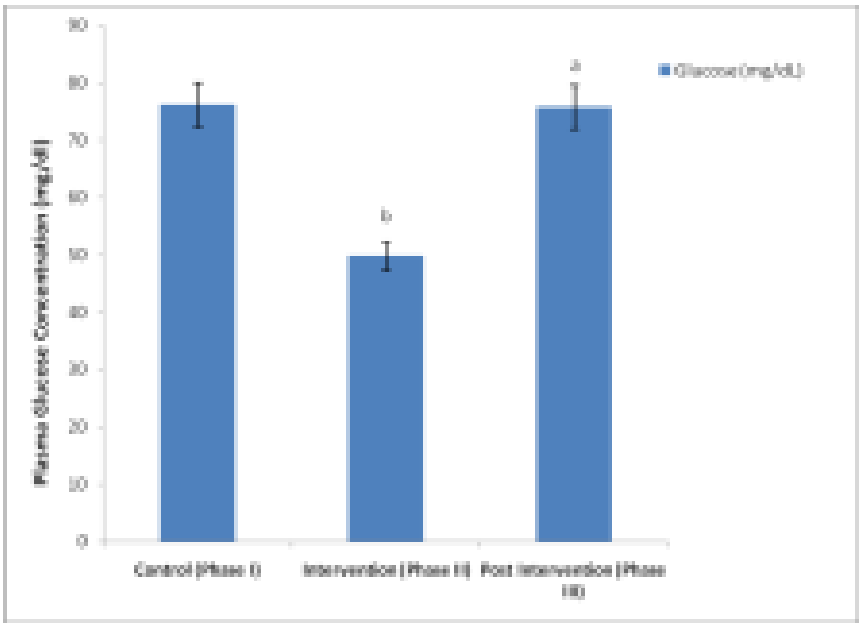

Figure 1: Effect of beetroot juice on plasma glucose of healthy subjects.
Values with different letters are significantly different (P < 0.05).
There was a significant decrease (P<0.05) in the plasma glucose of phase II when compared to phases I and III.

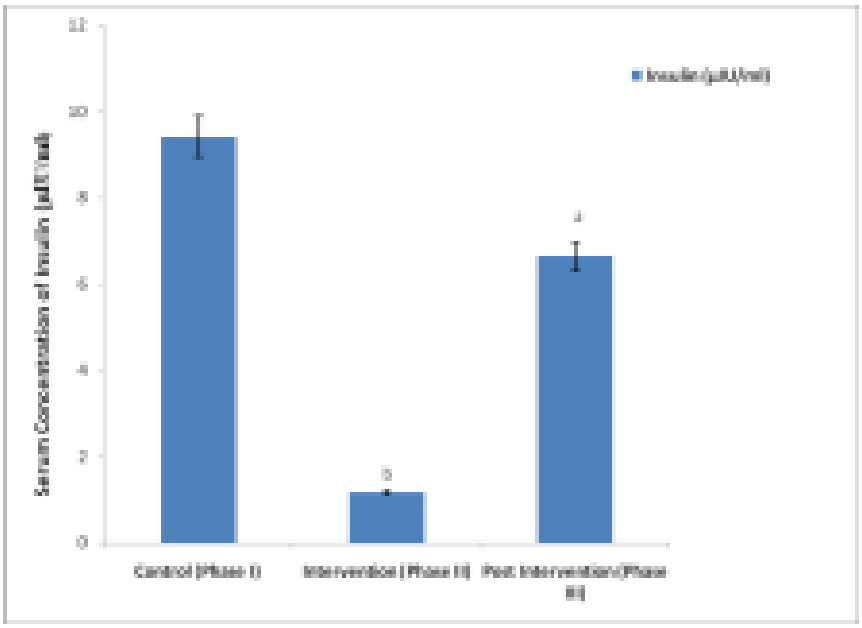

Figure 2: Effect of beetroot juice on serum Insulin in healthy subjects.
Values with different letters are significantly different (P < 0.05). The serum insulin level was significantly reduced (P<0.05) in phase II when compared to phases I and III

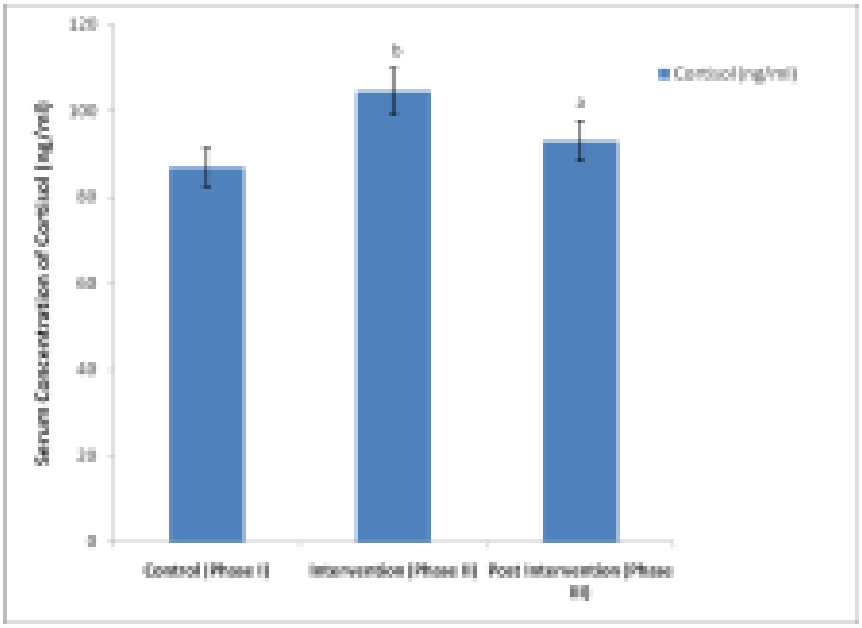

Figure 3: Effect of beetroot juice extract on serum cortisol in healthy subjects.
Values with different letters are significantly different (P < 0.05). The serum cortisol was significantly increased (P<0.05) in the intervention (phase II) when compared to control (phase I) and post intervention (phase III).

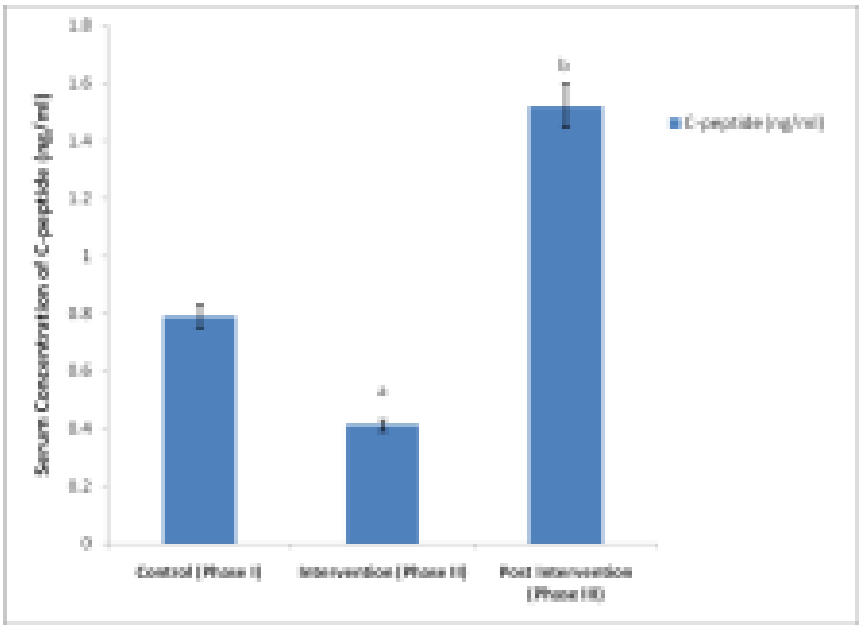

Figure 4: Effect of beetroot juice on serum C-peptide in healthy subjects.
Values with different letters are significantly different (P < 0.05). There was a significant decrease (P < 0.05) in serum C-peptide in phase II when compared to phases I and III.

Table 2. Correlation of Serum glucose, Insulin, C-peptide and Cortisol levels in healthy subject

	Glucose	C-peptide	Human Insulin	Cortisol
Glucose	1	-0.123	-0.251	0.092
Cpeptide		1	.587**	0.385
Human Insulin			1	-0.119
Cortisol Test				1

**significant at 0.01 level

Table 2 represents the correlation table between the variables (glucose, C-peptide, human insulin and cortisol) assayed to define the effect of consuming Beetroot juice.

The above correlation table showed that; the values obtained for human insulin (*r = -0.251; P0.05*) and C-peptide (*r = -0.123; P0.05*) have negative relationship with glucose that is not significant. However, there is a significant positive relationship (*r = 0.587; P<0.01*) between C-peptide and human insulin. Cortisol and human insulin have a negative relationship (*r = -0.119; P0.05)* while C-peptide and cortisol showed a positive relationship (*r= 0.385; P 0.05*). Glucose and cortisol showed weak relationship (*r= 0.092; P 0.05*).

DISCUSSION

The results of this study showed that the Beetroot juice has a rich content of polyphenols and flavonoids. Nigerian Beetroot has been reported to possess antioxidant activities[19]. Our findings are in agreement with earlier reports[10,11].

Current trends in bioactive compounds of plant species have shown that increasing levels of anthocyanins, flavonoids and polyphenols in the diet could decrease the occurrence of certain human diseases[20,21]. These polyphenols are very popular because of their anti-hyperglycaemic effects, safety and non side-effects. Potential efficacy of polyphenols on carbohydrate metabolism and glucose homeostasis has been well investigated *in vitro* and with animal models and some clinical trials carried out to determine their efficacies[22]. Literature suggests that polyphenols-rich natural products may offer unique treatment modalities for various aspects of type 2 diabetes[23]. These includes inhibition of α-amlylase and α-glucosidase, increase in paraoxonase 1 (PON1) activity, modification of intracellular signal transduction which is a major mechanism of reducing blood glucose by foods[24].

The significantly ($p<0.05$) increased serum cortisol level coupled with the reduced ($p<0.05$) glucose concentration in the intervention phase (consumption of beetroot juice) when compared to the control and post intervention phases could be that the reduction in blood glucose stimulated the release of cortisol as a counter regulatory mechanism. The correlation studies revealed a weak relationship between glucose and cortisol.

Cortisol, a stress hormone has been reported to increase gluconeogenesis, decrease peripheral glucose utilization[25] and increase the availability of fuel substances by the mobilization of glucose [26,27], free fatty acids [28], and amino acids from endogenous stores [29,30]. However, the results from this study showed that cortisol correlated negatively with insulin.

In this study the insulin and C-peptide concentrations in the intervention phase were significantly ($p < 0.05$) decreased compared to the control and post intervention periods. The hepatic effects of cortisol appears to be mediated by the inhibition of insulin and stimulation of glucagon [27]. *In vitro* studies have demonstrated that very high cortisol concentrations can inhibit insulin synthesis and secretion[31]. The results obtained in this study with human subjects are in agreement with those reported earlier. Insulin is produced by the beta-cells of the pancreatic islets and a human pancreas contains approximately 1 million pancreatic islets that are distributed throughout the exocrine parenchyma of the gland [32].

The increase in cortisol concentration by the beetroot juice could either be due to the activation of ACTH secretion in pituitary glands or the mode of action at the level of the adrenal cortex. It is likely that the reduced glucose concentration obtained in the intervention phase when beetroot juice was administered may not be insulin dependent. This was also reinforced from the results showing that insulin and C-peptide correlated negatively with glucose.

Separately considering serum C-peptide and blood glucose may not explain the hypoglycaemic effects observed, but rather on comparing with insulin. C-peptide is a product of proinsulin cleavage generated in pancreatic beta cells as a part of normal insulin production. It is released into the blood stream in equivalent amounts as insulin in response to various stimuli including elevated blood glucose [33]. C-peptide has insulinomimetic effects by triggering insulin receptor activity, and increases glycogen synthesis and amino acid uptake, but has no glucose lowering effect [34]. This is in line with the results of the correlation studies which showed a significant positive relationship between C-peptide and human insulin. The C-peptide and cortisol were weakly correlated. Literature has paucity of information on the measurement of serum C-peptide in patients with hyperglycaemic emergency. C-peptide measurement provides an accurate assessment of residual beta-cell function, and thus has been widely used as a marker of insulin secretion in patients with diabetes [35,36].

The hypoglycaemic activity of beetroot juice as clearly established by this study may not be dependent on insulin secretion. The possible mechanisms could include inhibition of carbohydrate digestion and glucose absorption in the intestine, and the modulation of glucose release from the liver.

CONCLUSION

Beetroot juice administration for six weeks to healthy subjects showed a glucose-lowering effect. Although we were not able to determine the precise mechanism of the glucose-lowering effects in this present study, the hypoglycemic effects may not be insulin dependent or dependent on inhibition of glucose absorption. Further study may reveal the precise mechanism underlying the glucose-lowering effect of Beetroot. Clinical studies with defined diets, controlled study designs and clinically relevant end points are essential to clarify the potential benefits of Beetroot in reducing blood glucose levels.

ACKNOWLEDGMENTS

The authors would like to thank TETFund for providing financial assistance in the execution of this research project.

REFERENCES

1. International Diabetes Federation (IDF). Global diabetes scorecard-tracking progress for action. Brussels, 2014.
2. Guyton AC, Hall JE. Textbook of Medical physiology. 11th Edition. Elsevier Inc, New Delhi, 2006
3. Eisenbarth GS, Buse JB. Type 1 diabetes mellitus. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, eds. Williams Textbook of Endocrinology . 12th ed. Philadelphia, PA: Saunders Elsevier; 2011:chap 32.
4. Buse JB, Polonsky KS, Burant C. Type 2 diabetes mellitus. In: Melmed S, Polonsky KS, Larsen PR, Kronenberg HM, eds. Williams Textbook of Endocrinology . 12th ed. Philadelphia, Pa: Saunders Elsevier; 2011;chap 31.
5. Martynyuk L, Ruzhitska O, Martynyuk O. Effect of the herbal combination Canephron N on Diabetic Nephropathy in patients with Diabetes Mellitus: Results of a comparative Cohort study. The J. Alt. Compl. Med 2014; 20: 472-478.
6. American Diabetes Association. Standards of medical care in diabetes. Diabetes Care 2014; 34 (S14-S80) Blonde L
7. Pencek R, MacConell L. Association among weight change, glycaemic control, and markers of cardiovascular risk with exenatide once weekly: a pooled analysis of patients with type 2 diabetes. Cardiovasc Diabetol 2015; 3(14):12 Patel
8. DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed. 2012; 2(4): 320–330.
9. Kazeem MI, Davies TC. Anti-diabetic functional foods as sources of insulin secreting, insulin sensitizing and insulin mimetic agents. J. Funct. Foods. 2016; 20:122 138.
10. Wootton-Beard PC, Ryan L. A beetroot juice shot is a significant and convenient source of bioaccessible antioxidants. J. Funct. Foods. 2011; 3: 329–334
11. Kavalcová P, Bystrická J, Tomáš J, Karovičová J, Kovarovič J, Lenková M . The Content of Total Polyphenols and Antioxidant Activity in Red Beetroot. Potravinarstvo 2015; 9(1): 77-83.
12. Clifford T, Howatson G, West DJ, Stevenson EJ. The potential benefits of red beetroot supplementation in health and disease. Nutrients 2015; 7(4): 2801-22.
13. Zhang Q, Pan J, Wang Y, Lubet R, You M.. Beetroot red (betanin) inhibits vinyl carbamate- and benzo(a)pyrene-induced lung tumorigenesis through apoptosis. Molecular Carcinogenesis 2013; 52(9): 686–691
14. Vulić JJ, Ćebović TN, Čanadanović-Brunet JM, Ćetković GS., Čanadanović VM, Djilas SM, Tumbas Šaponjac VT.. *In vivo* and *in vitro* antioxidant effects of beetroot pomace extracts. J. Funct. Foods 2014; 6: 168–175.
15. Singleton VL, Orthofor R, Lamuela Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocaltau reagent. Methods Enzymol. 1999; 299: 152 – 178
16. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Faso honey, as well as their radical scavenging activity. Food Chem. 2005; 91: 571–577.
17. Barham D, Trinder P. Improved color reagent for determination of blood glucose by oxidase system. Analyst 1972; 97: 142–145.
18. Bonger A, Garcia-Webb P. C-Peptide Measurement: Methods and Clinical Utility. CRC Critical Reviews in Clinical Laboratory Sciences 1984; 19: 297
19. Olumese FE, Oboh HA. Antioxidant and Antioxidant capacity of raw and processed Nigerian Beetroot (*Beta vulgaris*). Nigerian Journal of Basic and Applied Science 2016; 24: (2) (In Press) Bahadoran
20. Z, Mirmiran P, Azizi F. Dietary Polyphenols As Potential Nutraceuticals In Management Of Diabetes: A Review. J Diabetes Metab Disord. 2013; 12: 43.
21. Farid HEA, EL-Sayed SM, Abozid MM. Pumpkin and Sunflower Seeds Attenuate Hyperglycemia and Protect Liver in Alloxan-Induced Diabetic Rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences 2015; 6(5): 1269-1279
22. Hanhineva K, Törrönen R, Bondia-Pons I, Pekkinen J, Kolehmainen M, Mykkänen H, Poutanen, K. Impact of dietary polyphenols on carbohydrate metabolism. Int J Mol Sci. 2010; 11(4):1365–1402..Dragan S
23. Andrica F, Serban MC, Timar R. Polyphenols-rich natural products for treatment of diabetes. Curr Med Chem. 2015; 22(1):14-22
24. Uchida-Maruki H Inagaki H, Ito R, Kurita I, Sai M, Ito T. Piceatannol Lowers the Blood Glucose Level in Diabetic Mice. Biol. Pharm. Bull. 2015; 38: 629–633
25. Ganong WF. Review of Medical Physiology 17th edn., Appleton and Lange, Connecticut, 1995; p. 290–305
26. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. J Clin Endocrinol Metab. 1982; 54: 131–138
27. Dinneen S, Alzaid A, Miles J, Rizza R. Metabolic Effects of the Nocturnal Rise in Cortisol on Carbohydrate Metabolism in Normal Humans. J. Clin. Invest.1993; 92: 2283-2290
28. Djurhuus CB, Gravholt CH, Nielsen S, Mengel A, Christiansen JS, Schmitz OE, Møller N. Effects of cortisol on lipolysis and regional interstitial glycerol levels in humans. Am J Physiol Endocrinol Metab. 2002; 283: E172–E177
29. Horber FF, Haymond MW. Human growth hormone prevents the protein catabolic side effects of prednisone in humans. J Clin Invest. 1990; 86: 265–272
30. Berneis K, Ninnis R, Girard J, Frey BM, Keller **U.** Effects of insulin-like growth factor I combined with growth hormone on glucocorticoid-induced whole-body protein catabolism in man. J Clin Endocrinol Metab.1997; 82:2528–2534

31. Pierluissi J, Navas FO, Ashcroft SJH. Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans. Diabetologia 1986; 29: 119-121.
32. Rorsman P. Insulin Secretion: Function and therapy of Pancreatic beta-cells in diabetes. Brit. J. Diab. Vasc. Dis. 2005; (5)4: 187-191.
33. Tonoli C, Heyman E, Roelands B, Buyse L, Piacentini MF, Berthoin S, Meeusen R. Diabetes Associated Cognitive Decline, is there a Role for Exercise? J Diabetes Metab. 2013; S10 : 006. doi:10.4172/2155-6156.S10-006
34. Sima AA, Kamiya H, Li ZG. Insulin, C-peptide, hyperglycemia, and central nervous system complications in diabetes. Eur J Pharmacol. 2004; 490: 187-19
35. Gerard SK, Rhayam-Bashi H. Characterization of creatinine error in ketotic patients: a prospective comparison of alkaline picrate methods with an enzymatic method. Am J Clin Pathol. 1985; 84: 659–661.
36. Palmer JP, Fleming GA, Greenbaum CJ, Herold KC, Jansa LD, Kolb H, Lachin JM, Polonsky KS, Pozzilli P, Skyler JS, Steffes MW. C-peptide is the appropriate outcome measure for type 1 diabetes clinical trials to preserve β - cell function. Report of an ADA Workshop, 21–22 October 2001. Diabetes 2004; 53: 250–264.