International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Effect of Vitamin C and D*ialium guineense Fruit Pulp Activity* on HO-1, Insulin Secretion and Ace in Experimental Diabetes

Etah Nkanu[1], Victoria Okon[2], Ujong Gabriel[3], Esther George[4]

Department of Physiology, Cross River University of Technology

[1, 4]Department of Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Okuku Campus, Yala, Nigeria

Abstract : ***The study was targeted at evaluating the modulatory effect of aqueous fruit pulp of Dialium guineense on hemeoxygenase, insulin, angiotensin converting enzyme (ACE) and possible hypoglycemia in streptozotocin-induced diabetic rats. Twenty four male Wistar rats were randomly divided into four groups: control, diabetic control (Dm), diabetic + 300 mg/kg body weight Dialium guineese (Dm+ DG) and diabetic + 500 mg/kg Vitamin C (Dm +Vit. C).Animals were made diabetic by injecting a single dose of 55 mg/kg streptozotocin intraperitoneally. Dialium and Vitamin C were administered orally for three weeks. Result showed significant (p<0.01) increase in serum ACE and blood glucose level and a decreased(p<0.01)bilirubin, conjugated bilirubin, albumin, globulin, HO-1 and insulin level in the diabetic control group. There was a significant increase (p<0.01) in HO-1, insulin concentration and a significantly reduced blood glucose level in the Dialium guineense treated group when compared to Vitamin C group. Dialium and Vitamin C treated groups showed no difference in ACE level, Bilirubin, Albumin, and globulin concentration respectively. In conclusion, this study indicates that Dialium guineense fruit pulp has a hypoglycemic property, enhances HO-1 and insulin release than vitamin C but with a common activity level on ACE inhibition.***

Keywords: Hemeoxygenase, angiotensin converting enzyme, Dialium guineense, Diabetes., Vitamin C

1. Introduction

Interest in the management of diabetes mellitus has been on the increase for decades now. There are many evolving approaches to tackling this menace. Apart from the use of conventional drugs, nutritionist have also considered dietary care as one outstanding measure towards the management of this metabolic syndrome while others are redirecting their attention to the use of medicinal plants to treat most chronic diseases including diabetes and cardiovascular diseases because of its nutritional value and medicinal properties [1,2].

Research has shown that Nutrients such as vitamins are essential to cardiovascular health, and many other physiological functions, their deficiencies contribute to the high prevalence of metabolic syndrome, and diabetes mellitus. For instance, unusually Low levels of vitamin C in cells occurring as a result of hyperglycemia/glucose-induced oxidative stress has been associated with increased risk of the development of diabetes[3,4] Its dietary supplementation over time as well as other dietary sources including fresh fruits and vegetables have been reported to be of biological significance given their antioxidant status and ability to prevent diabetes. In addition to the enormous debilitating factors linked to the development of diabetes such *as* derangement of pancreatic β-cell that may lead to abnormal insulin secretion or insulin receptor inactivity, hyperglycemia, hypercholesterolemia, hemeoxygenase, bilirubin, albumin, and angiotensin converting enzyme levels become greatly altered leading to varied physiological dysfunction.

Reports have shown that even though bilirubin was initially seen as a waste productof the heme catabolic pathway and a potentially toxic compound for decades, it has some element of biological significance and is assumed to be one major compound that add to the total antioxidant status in blood plasma [5] . Slight elevation in serum bilirubin level often protects lipids from oxidation than glutathione [6,7]and reduces the prevalence of oxidative stress-mediated diseases such as arterial hypertension, diabetes mellitus, metabolic syndrome and cardiovascular disease[8]

Similarly, Heme oxygenase-1, an enzyme that breaks down heme moiety to produce carbon monoxide (CO), bilirubin and biliverdin [9,10]' all of which have the potential to suppress inflammation, and oxidative stress[11,12,13,14] , as w ell as enhancing insulin release and sensitivity in different rats strains[15,16,17,18]and potentiate insulin sensitivity and glucose metabolism, demonstrates antioxidant property.

Studies have also shown that natural ACE inhibitors like polyphenols, flavonoids, xanthines, and terpenoids derived from plant[19,20],and AT2 receptor blockers significantly decreased the risk of DM[21,22]. ACE, converts inactive angiotensin 1 to active angiotensin II which potentiates vasoconstriction and increase blood pressure. In a bide to establish the role of plants and their products in the management of diabetes, it becomes imperative to provide reliable information about the efficacy of such plant or its active principles [23].

Dialium guineense, otherwise referred to as black velvet tamarind has been reported to be used in the management of various disease conditions such as jaundice, antiulcer, severe cough, bronchitis, stomach aches, malaria fever,

hypertension and hemorrhoids[24] Essentially, the Phytochemical components identified in the sticky pulp of *Dialium guineense* include mucilage, pectin, tannins alkaloids, saponins, flavonoid, steroids, cardiac glycosides and some phenolic compounds[25,26]It also contains ascorbic acid, minerals like copper, potassium, calcium ,iron, selenium, zinc and magnesium. Vitamins found in this fruits include vitamin-A, folic acid, riboflavin, niacin and vitamin C, tartaric acid (an anti-oxidant), carbohydrates in the form of soluble sugars, cellulose, iron and lipids[27, 28]

This study was aimed at investigating the possible similar effects of Dialium and Vitamin C on the modulation of hemeoxygenase, insulin release and ACE inhibition in streptozotocin-induced diabetic Wistar rats.

2. Materials and Methods

Experimental Design

Twenty male Wistar rats weighing between 200-250g were used for this study. The animals were selected into four groups of six rats each. Group 1(Control), received tap water. Group 2, 3 and 4 were injected with 55 mg/Kg body weight of streptozotocin intraperitoneally to induce diabetes. Groups 3 and 4 were then administered 300 mg/kg of aqueous Dialium guineense fruit pulp and 500 mg/kg body weight of Vitamin C respectively. Administration of drugs lasted for three weeks. Blood samples were collected by cardiac puncture for biochemical analysis.

Preparation of Dialium fruit pulp extract

The fruit pulp of Dialium guineense was purchased from Okuku market, Yala Local Government Area of Cross River State, Nigeria. *Dialium guineense* fruits were collected and the dark coloured hard coats broken to expose the soft pink pulp of the fruit .The pulp were peeled from the water proof-like coat and then dried at room temperature by hot air oven (Amstel Hearson Oven, England). Dried pulp was blended to powder and used when necessary. Animals received 300 mg/kg body weight of the suspension

Induction of diabetes mellitus

The rats in groups 2,3 and 4 were rendered diabetic after a 12 hour fast by intraperitoneal injection of a single dose of 55 mg/kg body weight streptozotocin (SantaCruz Biotechnology, USA) dissolved in 0.01M sodium citrate buffer, at a pH 4.5. Absolute compliance with ethical guideline for research, care, and use of laboratory animals was adhered to. After 3 days of streptozotocin injection, blood glucose concentrations were determined via AccuChek glucometer to confirm diabetes. Blood glucose levels above 130mg/dl were considered.

Blood Sample collection and analysis

Blood samples were collected from the animals through cardiac puncture into ethylenediamine tetra-acetic acid (EDTA) bottles. The blood samples were centrifuged at 3000 (rpm) revolutions for 10 minutes to obtain serum for bilirubin, albumin, insulin, angiotensin converting enzyme and hemeoxygenase-1 analysis.

Biochemical measurements

Plasma HO-1(Assay designs, Michigan, US) and ACE (USCNLife, Wuhan, CN) activities were analysed using commercially available kits. Measurements were performed using modified method of Leong et al, 2010 according to the manufacturers instruction. The coloured end products of HO-1 and ACE were measured in microplate reader at 450nm.

Bilirubin and albumin were determined using standard methods as decribed by Grant and Kacchman,[30,31]. Briefly, 0.95 mL of sodium cholate phosphate buffer and add 50 j.tL of serum or bilirubin standard, (in serum albumin) were mixed and absorbance at 460 nm was recorded. 10 ILL of bilirubin oxidase (initial concentration, before addition, 70 mg/L) was added and mixed quickly. Absorbance change for 3 mm was recorded. From the standard curve of total bilirubin standards after 3 mm vs their initial concentrations, total bilirubin concentration of the test specimens was estimated by comparing their absorbance after 3 mm with the reference curve.

Statistical analysis

All data presented in this study were expressed as mean ± standard error of mean (Mean ± SEM). Analysis of variance (ANOVA) was used to analyzed collected data followed by Bonferronis multiple comparison post hoc tests to compare the level of significance between control and experimental groups. A value of $p<0.05$ was considered significant. All analysis were performed using the graph pad version 5 statistical software program

3. Result

Effect of Dialium guineense fruit pulp and Vitamin C on Biochemical substances in STZ-induced diabetic rats

Results obtained showed a significant ($p<0.05$) decrease in bilirubin and conjugated bilirubin levels in the diabetic group when compared to control and the treated groups. Albumin level was not significantly different in the diabetic and the treated groups, where as it was observed to be significantly($p<0.05$) higher in the three groups than control. Globulin concentration dropped in the diabetic group significantly($p<0.005$) but did not show any significant difference in control and the treated groups.

Effect of Dialium guineense fruit pulp and Vitamin C on Blood glucose concentration in STZ-induced diabetic rats

Serum blood glucose level in the diabetic group was significantly ($p<0.001$) raised at the end of the three weeks compared to the control and treated groups. Dialium guineense and Vitamin C groups recorded a significant decrease in blood glucose level but with a higher hypoglycemic effect in the Dialium guineense treated (66.54%) group when compared to Vitamin C(50.75%). This is shown in figure four.

Table 1: Table showing effect of Dialium guineense fruit pulp on bilirubin, albumin and globulin in streptozotocin induced diabetic rats

Variable	Control	DM	DM + DG	DM+VIT. C
Bilirubin	53.28±0.65	38.76±1.56**	42.22±1.59**[a]	41.33±1.24
Conjugated Bilirubin	43.50±1.07	20.50±0.78**	24.54±0.62**[a]	25.27±0.57
Albumin	22.00±0.707	27.40±0.81**	27.80±0.66**	28.43±0.55
Globulin	29.60±0.74	25.00±0.94**	29.40±0.87[a]	28.21±0.72

*Values are expressed in mean ± SEM, **=P<0.01 Vs Control, a=p<0.01 Vs DM, b=p<0.01 Vs DM+Vit C; n=5,*

Table 2: Showing effect of aqueous Dialium guineense fruit pulp and Vitamin C treatment on serum blood glucose level in streptozotocin-induced diabetic rats

	Control	DM	DM + DG	DM+VIT. C
Start	68±1.5	310.8±11.66 ***b,c	276.45±14.2***a,c	264.0±14.6
Wk 1	65.50±4.3	300.78±30.3	220.17±11.83	215.5±23.5
Wk 2	66.61±2.0	250.8±20.40	120.00±4.6	190.8±14.4
Wk 3	67.25±1.40	230.54±10.5***b,c	92.5±5.3	130.6±15.0***a,b
% decrease	1.47%	25.82%	66.54%	50.75%

Values are expressed in mean ± SEM, ***=P<0.01 Vs Control, a=p<0.01 Vs DM+DG b=p<0.01 Vs DM+Vit C n=5

Effect of Dialium guineense fruit pulp and Vitamin C on Haemeoxygenase,, ACE and Insulin concentration in STZ-induced diabetic rats

Figure 1 & 2 shows HO-1 and Insulin concentration in diabetic rats treated with Dialium and Vitamin C respectively. HO and insulin levels were significantly reduced in the Dm group compared to control and the treated groups. Treatment with Dialium and Vitamin C significantly ($p<0.01$) elevated HO-1 and insulin concentrations. Angiotensin converting enzyme (ACE) concentration was significantly raised in the diabetic control group. Treatment with Dialium and Vitamin C respectively, significantly decreased ACE concentration.

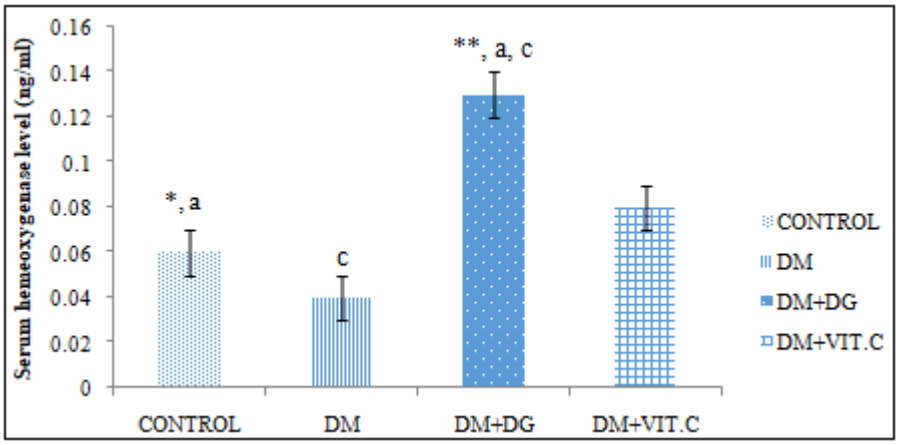

Figure 1: Serum hemeoxygenase concentration in control, diabetic control and treated groups. n=5, values are in mean ± SEM, ***=P<0.001 Vs Control, a=p<0.001 Vs DM, b=p<0.01 Vs DM+Vit C

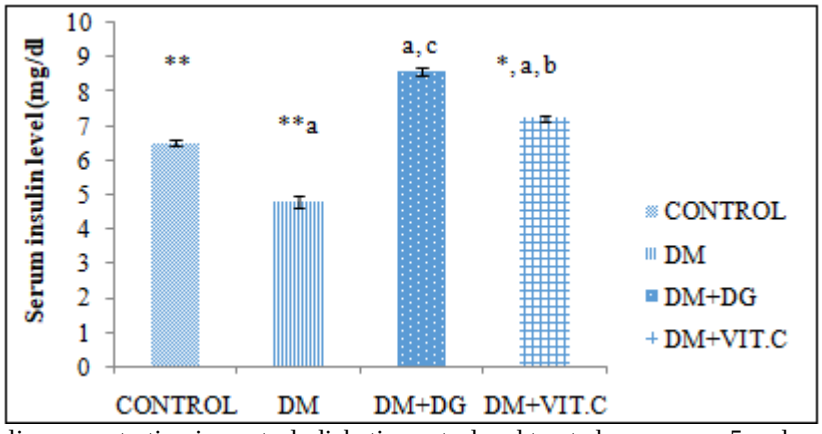

Figure 2: Serum insulin concentration in control, diabetic control and treated groups. n=5, values are in mean ± SEM, ***=P<0.001 Vs Control, a=p<0.001 Vs DM, b=p<0.01 Vs DM+Vit C

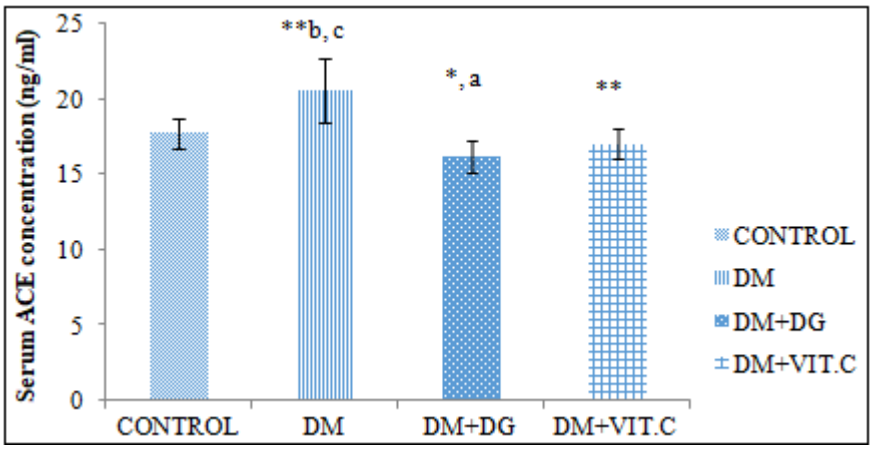

Figure 3: Serum angiotensin converting enzyme concentration in control, diabetic control and treated groups. n=5, values are expressed in mean ± SEM, ***=P<0.001 Vs Control, a=p<0.05 Vs DM,

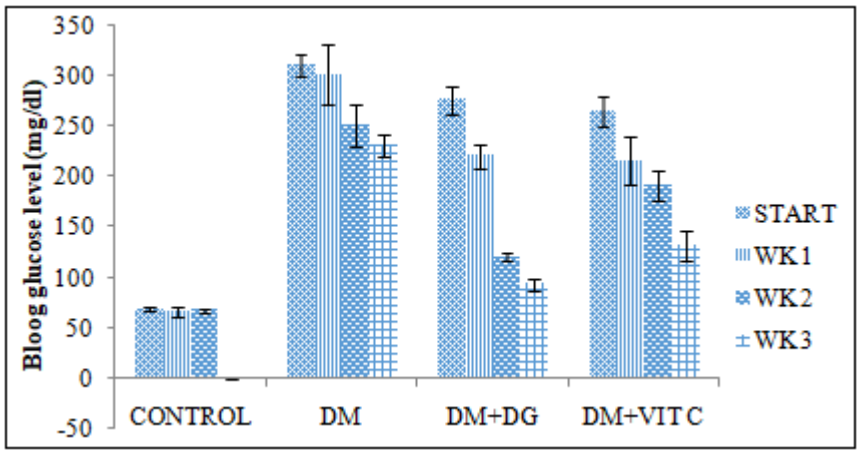

Figure 4: Showing fasting blood glucose level in control, diabetic control and treated groups. n=5, values are expressed in mean ± SEM, a=P<0.001 Vs Control, b=p<0.05 Vs DM

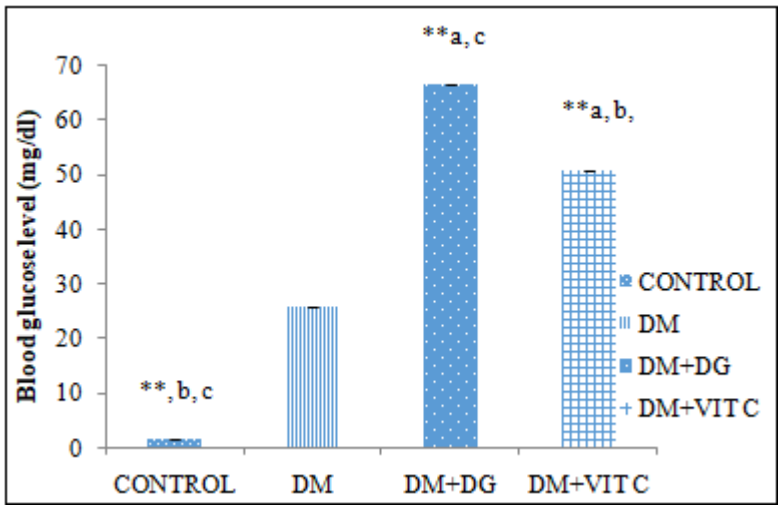

Figure 5: Showing percentage decrease in blood glucose level in STZ-induced diabetic rats treated with Dialium guineense fruit pulp and Vitamin c . n=5 **=p<0.01 vs control; a=p<0.05 vs Dm;b=0.05 vs Dm+DG;c=p<0.01 vs Dm+Vit c

4. Discussion

Hyperglycemia or elevated blood glucose level is characterized by oxidative stress and inflammatory insults, reduced vitamin C and formation of glycation end product of protein[32].This activity which often cause a break down in defense mechanism due to free radical production suppress the generation of carbon monoxide(CO) from the pancreatic beta cells of the Islets of Langerhans, bilirubin/biliverdin from heme catalytic pathway thereby promoting the tendency for inflammation and apoptosis[33,34]

From our study, we noted an increase in blood sugar level (Fig.5)and an appreciable decrease in bilirubin (Table1), hemeoxygenase and insulin release in diabetic rats, a situation often occurring as a result of elevated reactive oxygen species concentration in circulation. When free radical level increases beyond normal it leads to a geometric

progression of pancreatic beta cell depletion and insulin level, loss of bilirubin, HO-1 and globulin, hence contributing to the breakdown of immune system and to the pathogenesis of diabetes [35]

Bilirubin, apart from its early recognition as a toxic compound, clinical studies have shown that it contributes to the building up of antioxidant capacity in blood plasma[5] reduces the risk of diabetes mellitus and prevents coronary artery disease and atherosclerosis[36,37,38]. Bilirubin is formed from biliverdin by the actions of biliverdin reductase following the activity of hemeoxygenase (HO-1) enzyme that splits heme into biliverdin, CO, and ferrous iron. Similarly, HO-1 and CO present naturally in the pancreatic cells promote insulin release/sensitivity and glucose metabolism[19,20]as well as abating free radical production and immune/inflammatory response[39,40].

Our study has shown that plants may actually contain natural hemeoxygenase-1 inducers originating from plants polyphenols[41]. The oral administration of *Dialium guineense* fruit pulp and Vitamin C potentiated HO-1 and insulin release as well as increasing bilirubin production as demonstrated in figures 1and 2 and table 1 respectively . This results suggest a substantive reason for the observed hypoglycemia in this work. Under pathophysiological condition, HO-1 release may be minimal and temporal and serves as a starting point in the defense against oxidative insults and distortion of homeostasis. Therefore enhancing its further release by any means could help to attenuate the possible oxidative insults. Available evidence shows that Dialium guineense contains reasonable amount of ascorbic acid, vitamins A, and C, niacin, riboflavin tartaric acid[29,31] and flavonoid, alkanoid and phenolic compounds[25,26]all of which exhibit antioxidant/anti-inflammatory activity. While flavonoid serves as ACE inhibitor[20], niacin inhibits vascular inflammation via induction of HO-1[42] This could probably be the reason for the higher activity observed in *Dialium guineense* than vitamin C supplemented in this study.
Unlike HO-1 and insulin whose release was enhanced following DG administration, angiotensin converting enzyme activity both in Dialium and vitamin C supplemented groups were not different from one another. Both however, exhibited efficacy in decreasing ACE (Fig.3). This agrees with a previously reported work that tanins, flavonoids and phenols present in plants are responsible for ACE inhibition and angiotensin II (AT2) receptor blockade thereby reducing the risk of diabetes[21,22]. From the available results therefore, it is convincing that the antioxidant activity of Dialium guineense is more efficacious than that of vitamin C in the management of diabetes mellitus even though they both demonstrated therapeutic capacity.

Furthermore, our study revealed a reduced albumin concentration in diabetic animals and a significantly raised level in both Dialium and vitamin C treated groups. Albumin ordinarily is responsible for maintaining colloid osmotic pressure and micro-vascular integrity. It is clinically seen as a plasma expander and provides detoxification and protection by scavenging hydrogen peroxides, superoxide and hydrochloric acids. In pathological conditions where it becomes low it may lead to liver failure or chronic disorder. The increased level of albumin and globulin which is essential in cellular immunity recorded in this study following treatment with Dialium guineense fruit pulp and Vitamin C further suggest that they both have a strong bioactivity in ameliorating diabetes mellitus. .

5. Conclusion

This study clearly indicates that *Dialium guineense* fruit pulp and vitamin C have enhancing effects on hypoglycemia, HO-1 and insulin release but with a common activity level on ACE inhibition and Bilirubin/albumin protective function.

6. Acknowledgment

The authors of this research study wish to acknowledge the technical assistance of Dr. Mrs Iya Eze of the Department of Medical Laboratory Science, University of Calabar, Calabar, Nigeria.

7. Conflict of Interest

The authors declare that there is no conflict of interest concerning the publication of this paper.

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Author Profile

E. E. Nkanu is a Ph.D. holder and Head of Department of Physiology, Cross River University of Technology, Calabar, Okuku Campus, Cross River State. He has research interest in plant products and their cardiovascular effects with bias on ion channels.

Gabriel Ujonghas a Ph.D. in cardiovascular physiology and has a faculty position in Department of Physiology, Cross River University of Technology, Calabar, Okuku Campus, Cross River State, Nigeria. He has special interest in neurophysiology and cardiovascular Physiology.

Victoria Okon is a Ph.D. holder in Physiology with special interest in gastrointestinal tract and neurophysiology. She is employed in the Department of Physiology, Cross River University of Technology, Calabar, Okuku Campus, Cross River State Nigeria.

Esther George is a M.Sc. holder in Physiology with special interest in Endocrinology and Reproduction. She is employed in the Department of Physiology, Cross River University of Technology, Calabar, Okuku Campus, Cross River State Nigeria