ANTIDIABETIC EFFECT OF P*ERSEA AMERICANA* SEED EXTRACT IS MEDIATED THROUGH ENHANCED INSULIN SECRETION, IMPROVED BETA-CELL FUNCTION, AND REDUCED INSULIN RESISTANCE IN DIABETIC RATS

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ABSTRACT

There are reports of increasing prevalence of diabetes melitus worldwide. Also, few studies suggest the hypoglycaemic potentials of *persea americana* seed without assessing its effects on insulin, c-peptide, insulin resistance and beta-cell function in diabetics, hence the necessity for this study. Thirty (30) adult male wistar rats divided into 6 groups of 5 animals each were used. Group 1(normal control), 2 (diabetic control), 3 (extract only), 4, 5 and 6 (diabetes induced + extract 300mg/kg, 600mg/kg and glibenclamide, respectively), administered for 35 days. Fasting blood glucose (FBG) were assessed before alloxan induction of diabetes (day 0), 3 days after induction (day1), day 7 and day 35 respectively. On day 35, all animals were sacrificed, blood collected and serum used for analyses of insulin and c-peptide, while insulin resistance and beta-cell function were calculated from the FBG and insulin. Results show significant ($P<0.05$) increase in FBG on day 1 in the diabetes induced rats (groups 2,4,5 and 6). However, day 35, FBG significantly ($P<0.05$) reduced in the treated diabetic rats, compared to the diabetic control. Also, there was significant ($P<0.05$) decrease in insulin, c-peptide and beta-cell function, with significant ($P<0.05$) increase in insulin resistance, in the untreated diabetic rats, compared to the normal control. On the other hand, the insulin, c-peptide and beta-cell function significantly ($P<0.05$) increased, with a significant ($P<0.05$) reduction in the insulin resistance, in all the extract-treated and gibenclamide-treated diabetic groups, compared to the untreated diabetic control group. We therefore conclude that *persea americana* seed extract causes anti-hyperglycaemia in diabetic rats by enhancing insulin and c-peptide secretion and improving beta-cell function possibly through beta-cell regeneration, while reducing insulin resistance.

Keywords: *Persea americana,* anti-diabetic, insulin, beta-cell function, insulin resistance.

INTRODUCTION

Diabetes Mellitus, an endocrinological disorder resulting from impaired pancreatic beta-cell secretion of insulin and or impaired insulin action. This leads to disturbances in the metabolism

of carbohydrates, lipids, and proteins (Mahmoud, 2015; Daniel *et al.*, 2013; and American Diabetes Association, 2013) and persistent hyperglycaemia (Jahan *et al*., 2015). The prevalence of the disease is on the increase worldwide. The international diabetes foundation (IDF), projected that by year 2030 over 500 million adults will be affected. Though previously considered rare in sub-Saharan Africa (Sabir *et al*., 2017), studies have projected higher prevalence in Africa and Asia, where there is rapid epidemiological transition (King *et al*., 1998). Due to its increasing prevalence, and significant side effects seen in some pharmacological agents, such as sulfonylurea, used in its treatment (Parish and Saravanan, 2004), there is need for alternative natural anti-hyperglycemic agents with minimal side effects for the management of diabetes melitus, since most of the important drugs for the past 4 to 6 decades, which have revolutionized modern medicinal practice have been isolated or derived from plants (Dar *et al*., 2017).

Persea americana seeds have been reported to contain bioactive substances such as phenols, alkaloids, saponins and flavonoids (Ezejiofor *et al*., 2013, Okon *et al*., 2018), carbohydrate, Zinc, Iron, Manganese and Magnesium (Kawagishi *et al*., 2001). Studies have also suggested its cytotoxic and anti-tumor, antimicrobial, and anti-diabetic potentials (Dabas *et al*., 2013). However, most studies on its effects on diabetic animals are limited to assessing the blood glucose level, without assessing the insulin and c-peptide levels as well as the *B*-cell function and insulin resistance, hence the necessity of this study.

METHODOLOGY

Collection And Processing Of Pant Materials

Ripened fruits of *Persea americana* (Avocado pear) were collected at Rumuokoro market in Port Harcourt, Rivers State, Nigeria. It was identified and authenticated by taxonomists in the department of Plant Science and Biotechnology, University of Port Harcourt, and a Herbarium no. UP/UV/1395 was assigned. The seeds were removed and sliced into small particles with a knife, air-dried and then ground into powder. The powder was soaked in Hydro-methanol (30:70) solvent for 72 hours, and then filtered. The filtrate was concentrated by Rotary evaporator at 45^0C, and at 40 rotations per minute. The concentrate was then dried in a water bath and the resulting paste-like extract was preserved in a refrigerator at 4^0C, for use in this experiment.

Animal Models and Experimental Design

Thirty (30) adult male *Wistar* rats weighing between 170g and 200g were obtained from and managed in the animal house facility of the Faculty of Basic Medical Sciences, University of Port Harcourt. The animals were maintained under standard environmental conditions of temperature, relative humidity and dark/light cycle, and according to the guidelines and regulations of National Institute of Health (NIH) for the Care and Use of Laboratory Animals (NIH Publication, 85-23, 1985), and the Institutional Ethical committee of University of Port Harcourt. The rats were maintained in clean cages and were allowed free access to feeds and water *ad libitum*. After 2 weeks period of acclimatisation, the rats were weighed and randomly assigned into 6 groups of 5 rats each.

Group 1: Non-Diabetic Control administered 1ml water daily
Group 2: Diabetic Control (Alloxan induced diabetes)
Group 3: Extract treated (600mg/kg)

Group 4: Diabetes induced + Extract treated (300mg/kg)
Group 5: Diabetes induced + Extract treated (600mg/kg)
Group 6: Diabetes induced + Glibenclamide(0.5mg/kg)

Induction of Diabetes

Diabetes was induced in the rats (after over-night fast) by using intra-peritoneal injection of alloxan monohydrate (150 mg/kg) dissolved in normal saline immediately before usage (Venkatesh et al., 2010; Madhavan et al., 2008). Animals in groups B,D,E and F were induced,The normal control (nondiabetic) animals were injected with 0.5 ml of the vehicle (normal saline). Animals in all the groups were then provided with 5% dextrose water and feed, *ad libitum* for 48 hours. Diabetes was verified in the animals by measuring the fasting blood glucose 7 days following alloxan administration (Ragavan and Krishnakumari, 2006; Rajagopal and Sasikala, 2008), and were designated as day 1 of the study. Animals with FBG level >=15.00mmol/L were considered diabetic, and used for the study.

Collection of Blood Samples and Assay of Biochemical Parameters

Blood was obtained through a sterilized lancet prick to the tail of the animals, for determination of blood glucose using a digital glucometer (Accu-Check, Johnson-Johnson, Carlifornia, USA). On day 35, five animals from each group were sacrificed by cervical dislocation, and 4ml of blood aspirated by cardiac puncture and then emptied into plain sample bottles. Blood samples were centrifuged at 3000 rpm for 5 min and serum was stored frozen until needed for biochemical assays of insulin and C-peptide levels, using Enzyme-Linked Immunosorbent Assay (ELISA) method as described by Yalow *et al.,* (1960).

Determination of Fasting Blood Glucose (FBG)

Following an overnight fast, the blood glucose of each rat was determined at the commencement of the study before induction of diabetes (day0), then repeated after another 72 hours (Day1), day 7, and day 35 of the study.

Assessment of Insulin Resistance and Pancreatic β-cell Function

The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated with the formua : HOMA-IR = (fasting plasma insulin (mIU/L) × FPG (mmol/L)) divided by (22.5); while the homeostasis model assessment of pancreatic β-cell Function (HOMA-β) was calculated with the formula: HOMA-β = (20 × fasting plasma insulin (mIU/L)) / (FPG – 3.5); and as previously reported (Lee *et al.* 2016; Shashaj *et al*. 2016, and Wallace *et al.*2004).
The Relative changes for fasting blood glucose (FBG), insulin, c-peptide, insulin resistance and pancreatic β-cell Function (HOMA-β) respectively, were calculated by comparing diabetic control with normal control, and diabetic treated groups with the diabetic control group.

Statistical Analysis

Statistical analyses were performed by using the SPSS 12.0 statistical package for Windows (SPSS, Inc., Chicago, IL, USA). All values were expressed as mean±S.E.M. Statistical difference among the groups was assessed by one-way ANOVA with Dunnett's post hoc test. To compare data within the group, paired t-test (two-tailed) was performed. P values <0.05 were considered significant.

RESULTS:

Effect of Extract on Fasting Blood Glucose (FBG)

From the study, the blood glucose level (figure 1) in day1, shows a significant (P<0.001) increase for animals in the diabetes-induced groups, when compared to those in the normal control group. Thereafter, a progressive reduction in the blood glucose level was observed in the diabetic animals treated with the extract, or glibenclamide, when compared to the untreated diabetic control animals. By day 35, all the diabetic animals treated with 300mg/kg or 600mg/kg extract, or glibenclamide, respectively, showed significant (P<0.001) reduction in blood glucose levels, when compared to the untreated diabetic control animals; The percentage reduction in blood glucose for the treated diabetic animals (figure 2) was: 69.45% (300mg/kg extract), 60.69% (600mg/kg extract) and 75.60% (glibenclamide), respectively.

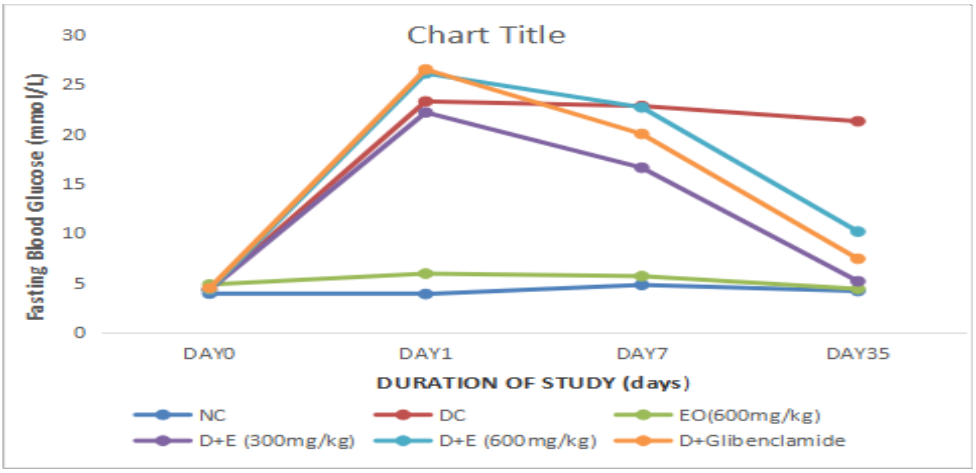

Figure 1: Effect of *Persea americana* seed extract on fasting blood glucose (FBG) level in diabetic rats.

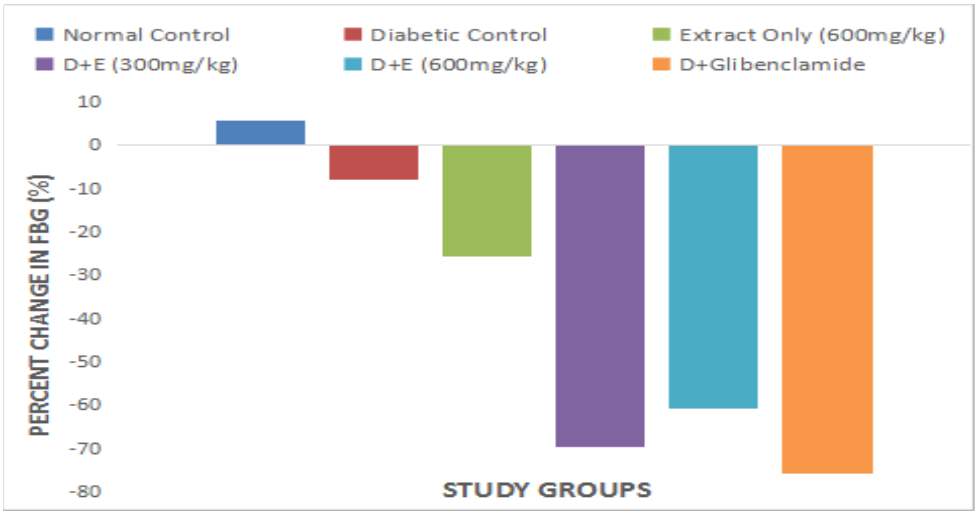

Figure 2: Percent Change in Fasting blood glucose levels following administration of *Persea americana* seed extract and glibenclamide in diabetic rats.

Effect of Extract on Serum Insulin and C-Peptide

The serum levels of Insulin and C-Peptide with their relative changes are presented in Table 1. Both insulin and c-peptide decreased significantly (P=0.001) in the untreated diabetic animals,

when compared to those in normal control group. However, treatment of the diabetic rats with extract significantly (P=0.001) increased the insulin level in the rats treated with 300mg/kg extract, 600mg/kg extract as well as glibenclamide respectively, compared to the untreated diabetic control group. Similarly, c-peptide level increased significantly (P=0.001) in the diabetic rats treated with 300mg/kg extract, 600mg/kg extract and glibenclamide respectively, when compared to those in the untreated diabetic control group.

Tabe 1: Effect of *persea americana* seed extract on serum insulin and c-peptide levels in diabetic rats

Study group	Insulin (mIU/L)		C-Peptide (pg/ml)	
	M±SEM	Relative Change (%)	M±SEM	Relative Change (%)
Normal Control	16.87±0.68	-	276.48±8.79	-
Diabetic Control	6.87±0.34[a]	- 59.28	143.96±11.90[a]	- 47.93
Extract (600mg/kg) only	14.77±1.01[b]	114.99	276.96±16.62[b]	92.39
D+ Extract (300mg/kg)	13.90±0.92[b]	102.33	222.60±13.73[b]	54.63
D+ Extract(600mg/kg)	12.27±0.86[b]	78.60	255.94±12.79[b]	77.79
D + G (0.5mg/kg)	14.71±0.47[b]	114.12	263.83±1098[b]	82.27

M±SEM = mean ± standard error of mean; D= diabetes induced; G = Glibenclamide.
[a](P<0.01 compared to normal control); [b](P<0.01 compared to diabetic control).

Effect of Extract on Insulin Resistance (HOMA-IR)

Results show a significant (P=0.001) increase in insulin resistance (Table 2) in the untreated diabetic rats, when compared to the normal control group. However, the insulin resistance decreased significantly in the diabetic rats treated with 300mg/kg extract (P=0.001), 600mg/kg extract (P=0.002) and glibenclamide (P=0.001), respectively, when compared to those in the untreated diabetic control group.

Effect of Extract on Pancreatic Beta-cell Functions (HOMA-β)

A significant (P=0.001) decrease in beta-cell function (Table 2) was observed in the untreated diabetic rats, compared to the non diabetic control. However, treatment of the diabetic rats with the extract or glibenclamide resulted to significant (P=0.001) increase in beta-cell function for all the extract-treated groups and the glibenclamide-treated group respectively, when compared to the untreated diabetic rats.

Tabe 2: Effect of *persea americana* seed extract on β-cell function and Insulin resistance diabetic rats.

Study group	Pancreatic Beta-cell Functions (HOMA-β)		Insulin Resistance (HOMA-IR)	
	M±SEM	Relative Change (%)	M±SEM	Relative Change (%)
Normal Control	390.86±22.95		3.20±0.17	
Diabetic Control	6.36±0.53[a]	- 98.37	7.73±0.41[a]	141.56
E (600mg/kg)	334.52±35.81[b]	5159.75	3.03±0.26[b]	- 60.80
D+ E(300mg/kg)	161.31±25.42[b]	2436.32	4.12±0.78[b]	- 46.70
D+ E (600mg/kg)	115.55±8.08[b]	1716.82	4.47±0.61[b]	- 42.17
D + G (0.5mg/kg)	125.54±25.27[b]	1873.90	3.91±0.14[b]	- 49.42

M±SEM = mean ± standard error of mean; D = diabetes induced; E = Extract; G = Glibenclamide.
[a](P<0.01 compared to normal control); [b](P<0.01 compared to diabetic control).

DISCUSSION

This study investigated the effect of hydromethanol extract of *Persea Americana* seed on pancreatic beta-cell function and insulin resistance in alloxan-induced diabetic rats. Alloxan, a cytotoxic diabetogenic compound, is widely used in experimental research studies of diabetes [Rohilla and Ali, 2012]. It stimulates insulin-dependent diabetes (T1D) by inducing selective necrosis of the beta-cells of pancreatic islets, thus, destroying β-cells and reducing their function [Siddique et al.,2013; Atanu and Momoh, 2018]. Our results showed that the hydro-methanol extract of *Persea americana* seed causes a significant ($P<0.05$) reduction in blood glucose levels in diabetic rats, comparable to glibenclamide, a known antidiabetic drug. This suggests anti-diabetic activity of *Persea americana* seed, thus agreeing with similar reports (Ezejiofor *et al.*, 2013; Aigbiremolen *et al*., 2017).

The present study also revealed that induction of diabetes led to significant reduction in the serum levels of insulin and c-peptide due to the destruction of the pancreatic b-cells, consistent with Gabr et al. (2015), while treatment with the extract enhanced the serum insulin and c-peptide levels in the treated diabetic rats. This suggests a possible cell regeneration activity of the seed extract on the damaged pancreatic β-cells (Zhong and Jiang, 2019), which function in the production and secretion of insulin, and c-peptide. Insulin promotes glucose uptake into cells and inhibit glucose release from the liver (Longnecker *et al*., 2014).

There was also an increase in insulin resistance in the untreated diabetic rats.The peripheral tissues of the body develop resistance when there is decrease sensitivity to insulin (Kahn *et al*., 2006), while insulin resistance (IR) is progressively coupled to onset of pancreatic β-cell dysfunction (Kim et al. 2018; Moon et al.2017 and Elochukwu et al. 2017, and King 2012), as observed in the untreated diabetic rats. Treatment with the extract significantly reduced the insulin resistance and the hypergycaemia, and therefore improved the beta-cell function. This suggests that the seed extract of *persea americana* possibly enhanced the insulin signalling pathways in the peripheral tissues necessary for improved glucose uptake and utilisation, which also contribute to the reduction in FBG and insulin resistance and eventually improvement in beta-cell function observed in the treated diabetic rats.

These antidiabetic effects of *persea americana* may be due to the rich content of calcium, magnesium, potassium, zinc and chromium in *persea americana* seed (Alhassan et al., 2012), which play important roles in blood glucose homeostasis by regulating the key enzymes involved in gluconeogenesis and enhancing glucose uptake and utilisation in the body. The enhanced beta-cell function, evidenced by the increased secretion of insulin and c-peptide, plus the reduction in insulin resistance may have all contributed to the observed reduction in blood glucose in the extract-treated diabetic rats.

CONCLUSION

This study has shown that the anti-diabetic effects of *Persea americana* (Avocado pear) seed extract in *wistar* rats is mediated through enhanced pancreatic secretion of insulin and c-peptide with improved beta-cell function, as well as reduction in insulin resistance. Therefore, if properly explored, the extract may be useful in the management of both insulin dependent (type 1) and non insulin dependent (type2) diabetes mellitus.

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