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Hydro-Ethanol Extract of *Persea americana* Fruit Pulp Ameliorates Dyslipidaemia and Cardiotoxicity Exerted by Alloxan-Induced Diabetes Mellitus

Bernard Omokheshi Adele[1], Chidi Okonkwo[1], Anthony Olusoji Odetola[1,2], Idara Emmanuel Emediong[1], Abayomi Oluwatosin Ige[1*], Elsie Olufunke Adewoye[1]

[1]Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan, Ibadan, Nigeria
[2]Department of Physiology, College of Health Sciences, Nnamdi Azikiwe University, Awka, Nigeria
Email: *aby_ige@yahoo.com, ao.ige@mail1.ui.edu.ng

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Abstract

Diabetes-induced dyslipidaemia has been associated with an increased risk of atherosclerosis and coronary heart diseases. *Persea americana* fruit has been reported to possess anti-diabetic properties. Therefore, this study assessed the lipid profile and likely cardio-protective effects of hydroethanolic extracts of *P. americana* fruits in alloxan-induced diabetic Wistar rats. Thirty-five male rats (150 ± 30 g) were divided into 5 groups (n = 7) and treated orally as follows; groups I-II were normal animals treated with distilled water (0.3 ml/day) and *P. americana* (300 mg/kg) only respectively. Animals in groups III-V were made diabetic using alloxan monohydrate (100 mg/kg *i.p.*) and treated orally with distilled water (0.3 ml/day), *P. americana* (300 mg/kg) and glibenclamide (5 mg/kg) respectively for 21 days. Fasting blood glucose level was monitored prior to, after induction of diabetes mellitus, and on day 21 post-treatment, respectively. Thereafter, retro-orbital blood samples were collected after anaesthesia and analysed for insulin, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL) levels, apolipoproteins A1 and B, superoxide dismutase (SOD) and catalase activities, reduced glutathione (GSH), Vitamin C and malondialdehyde levels, respectively. VLDL, atherogenic index (AI) and ApoB/A1 ratio were estimated mathematically. Pancreatic and cardiac structures were also investigated using Haematoxylin and Eosin stains. Treatment with *P. ameriacana* extracts reduced ($p < 0.05$) fasting blood glucose and increased ($p < 0.05$) insulin level compared to the diabetic untreated group. Values of TG, TC, LDL, Apo-B, AI, Apo-B/A1 ratio decreased significantly ($p < 0.05$) while

HDL increased significantly with extract treatment compared to diabetic untreated group. Serum malondialdehyde levels were significantly reduced in the extract treated diabetic group, while SOD and GSH significantly increased compared to diabetic untreated. Histological studies showed partial attenuation of diabetic-induced pathologies in the *P. americana* treated diabetic group. The hydro-ethanol fruit extract of *Persea americana* attenuates diabetes induced dyslipidaemia and reduces the susceptibility to cardiac impairment in experimental diabetes mellitus.

Keywords

Persea americana, Diabetes Mellitus, Dyslipidaemia and Alloxan

1. Introduction

Diabetes mellitus is a chronic metabolic disorder that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces [1]. Cardiovascular risk factors such as obesity, hypertension and dyslipidaemia are common in patients with diabetes mellitus and this places them at increased risk for cardiac events [2] [3]. In fact, cardiovascular diseases have been described as the major cause of morbidity and mortality (accounting for about 50% - 70% of deaths) in diabetic patients [4]. Hence, one of the primary goals in the management of diabetes mellitus, aside from glycaemic control, is to reduce the development of cardiovascular risk factors such as dyslipidaemia and hypertension in diabetic patients. Diabetic dyslipidaemia, a condition characterised by elevated fasting and postprandial triglycerides, reduced high density lipoprotein (HDL)-cholesterol, and elevated low-density lipoprotein (LDL)-cholesterol, is often described as one of the major risk factors for the development of cardiovascular diseases in diabetes mellitus [2] [5]. The lipid changes associated with diabetes mellitus have been attributed to an increase in the mobilisation of free fatty acid from adipose tissue stores secondary to insulin resistance [6].

Despite the availability of various pharmaceuticals designed to lower blood glucose level and ameliorate dyslipidaemia in diabetes mellitus, mortality from coronary heart disease arising from serum cholesterol levels has been observed to be increased [7] [8]. Aside from the undesirable side effects such as excessively low blood sugar, stomach upset, skin rash or itching, weight gain, kidney complications, bloating, diarrhoea etc, the high cost of these pharmaceuticals in low to middle income countries has led to a preference for hypoglycemics and serum cholesterol regulating agents (anticholesteremics) that are of plant origin [9] [10]. Furthermore, the phytochemicals in these medicinal plants (e.g., *Artemisia pallens*, *Bidens pilosa*, *Bixa orellana*, *Teramnus labialis*, *Cinnamomum zeylanicum*, *Croton cajucara*, *Allium spp.*, *Musa sapientum*, etc) have been observed to exert a multifactorial and multifaceted approach in the management of

diabetic mellitus [9].

One of such medicinal plants is *Persea americana*, commonly known as avocado, a flowering plant from the Lauracea family that is native to south central Mexico and cultivated in tropical and Mediterranean climes throughout the world [11]. Various parts of *P. americana* ranging from the leaf, seed, and fruit pulp have been extracted with different solvents and reported to be rich in bioactive compounds with anti-diabetic and antioxidant [12], hypolidaemic, anti-aging [13], and anti-cancer [14] activities. Its effect on dyslipidaemia and cardio-toxicity in diabetes mellitus is however yet to be fully elucidated. This study was therefore designed to evaluate the likely ameliorative potential of hydro-ethanol extract of *Persea americana* fruit pulp on dyslipidaemia and cardiotoxicity in diabetes mellitus using the alloxan-induced diabetic rat model.

2. Material and Method

2.1. Collection and Preparation of *Persea americana* Fruit Extract

Fresh fruits of *Persea Americana* were purchased from the local market in Ibadan, Nigeria and identified at the University of Ibadan Herbarium (UIH-22767). The fresh fruits were washed, peeled and seeds removed. The edible fruit pulp was chopped into small pieces, air dried for 24 hrs, and subsequently oven dried at 30˚C - 40˚C for 4hrs. Thereafter, the dried fruit pulp was ground into powder and 850g of it was weighed and macerated in 5000 ml of 70% ethanol for 72 hours with constant stirring. The hydro-ethanol filtrate was collected using a muslin bag and Whatman No. 1 filter paper and concentrated using a rotary evaporator. The hydro-ethanol extract of *P. americana* fruit obtained was then stored at 4˚C until use.

2.2. Animals and Induction of Diabetes Mellitus

Twenty-five male Wistar rats (150 ± 30 g) were housed in well-aerated laboratory cages, maintained on standard rat chow and water *ad libitum*. They were exposed to natural alternating day and night cycles in the animal house. Animals were kept in accordance with the ethics and guideline for the use of experimental animals in the laboratory of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) and that of the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). Experimental diabetes mellitus was induced using a single intra-peritoneal administration of alloxan monohydrate (100 mg/kg) to overnight fasted animals [15]. The blood glucose level of each experimental animals was thereafter monitored (glucose oxidase method) at intervals of 24 hours for 72 hours using the tail tipping method [16]. Animals that presented with relatively constant fasting blood glucose (FBG) values above 250 mg/dL were considered as being diabetic and recruited for the study [17].

2.3. Animal Grouping and Experimental Protocol

The animals were randomly grouped (n = 5) as follows; group I, control was

treated orally with distilled water (0.3 ml/day). Animals in group II were treated with *Persea americana* (300 mg/kg) only [12] while animals in groups III – V were made diabetic and treated with distilled water (0.3 ml/day), *P. americana* (300 mg/kg) and glibenclamide (5 mg/kg) [18], respectively. All treatments were carried out orally for 21 days using an orogastric tube.

2.4. Biochemical Assays

Blood samples were collected from the retro-orbital sinus after light anesthesia (ketamine: zylazine) using a heparinized capillary tube into plain sample bottles. The samples collected were allowed to stand at room temperature for 10 minutes and then centrifuged for 10 minutes at 3500 rpm. The clear serum obtained was analyzed for total cholesterol, high density lipoprotein (HDL), triglycerides, insulin, apolipoprotein A-1 (Apo A-1), and apolipoprotein B using commercially available laboratory kits. Low density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were estimated using Friedewalds equation [19] while atherogenic index was calculated using the formula = log(TG/HDL-C). Serum antioxidants (superoxide dismutase (SOD) [20], catalase [21], reduced glutathione (GSH) [22], ascorbic acid [23] and lipid peroxidation (thiobabituric acid reactive substances (TBARS) [24] were also analyzed in the serum samples.

2.5. Histological Studies

The heart and pancreas of each animal were also excised, fixed in 10% formalin and evaluated for structure and architectural integrity using Haematoxylin and Eosin staining techniques [25] [26].

2.6. Statistical Analysis

Data obtained are presented as mean ± standard error of mean (SEM) and statistical evaluation was carried out using Graph Pad Prism 7.0 (GraphPad Software Inc., USA). Statistical significance was assessed using one-way analysis of variance (ANOVA) and pairwise comparisons were conducted using Newman Keul post-hoc test.

3. Results

3.1. Effect of *Persea americana* on Fasting Blood Glucose Level in Control and Experimental Groups

Fasting blood glucose level (mg/dL) in group I, the control group, and group II (*P. americana* only) was relatively constant throughout the duration of the study. Values obtained in the groups III (75.8 ± 4.5 vs. 312.8 ± 15.7), IV (72.5 ± 8.2 vs. 305.4 ± 20.5) and V (68.8 ± 7.2 vs. 325.5 ± 15.5) on day 0 after the induction of diabetes mellitus were significantly increased ($p < 0.05$) compared to initial blood glucose values obtained in these experimental groups respectively. On day 21, values in group III where 12.0% reduced compared to values on day 0 while values obtained in groups IV and V were reduced by 52.4% and 65.39% respec-

tively compared to values obtained within these groups on day 0. Fasting blood values obtained in groups IV and V on day 21, though reduced compared to diabetic untreated (group II), were still increased ($p < 0.05$) compared to control (Table 1).

3.2. Effect of *P. americana* on Insulin Levels in Control and Experimental Groups

Insulin levels (μU/mL) at the end of the study were reduced in diabetic untreated group (group III) compared to group I (control) and group II (*P. americana* only) respectively. Values obtained in group IV (diabetic + *P. americana*) were significantly increased compared to groups I, II and III respectively. Insulin values obtained in group V (diabetic + glibenclamide) were comparable to group III and significantly reduced compared to groups I, II and IV respectively (Figure 1).

Table 1. Blood glucose level in control and diabetic treated groups.

	Groups				
	I	II	III	IV	V
	Fasting blood glucose levels (mg/dL)				
Initial values	70.5 ± 3.2	65.7 ± 7.5	75.8 ± 4.5	72.5 ± 8.2	68.8 ± 7.2
Day 0 (Post diabetic induction)	75.3 ± 4.1	70.4 ± 6.4	312.8 ± 15.7*	305.4 ± 20.5*	325.5 ± 15.5*
Day 21	72.8 ± 3.2	80.8 ± 5.2	275.5 ± 20.2*	145.5 ± 10.6*#	125.8 ± 10.5*#

Values are expressed as Mean ± SEM; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

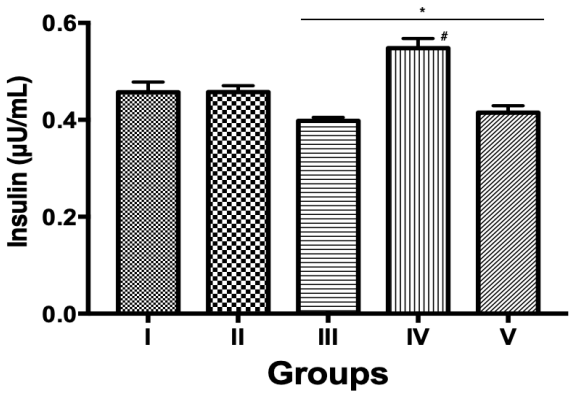

Figure 1. Insulin level in normal and diabetic treated groups. *Values are expressed as Mean ± SEM;* n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

3.3. Effect of *P. americana* on the Lipid Profile in Control and Experimental Groups

Evaluation of the lipid profile (all in mg/dL) shows a reduction ($p < 0.05$) in triglycerides (49.3%), total cholesterol (62.6%), high density lipoprotein (HDL) (63.2%), low density lipoprotein (LDL) (64.9%) and very low density lipoproteins (VLDL) (49.3%) in group II (*P. americana* only) compared to group I, the control group. Triglycerides (83.32 ± 0.23 vs. 77.92 ± 0.57), total cholesterol (119.14 ± 3.55 vs. 107.23 ± 2.44), LDL (100.25 ± 3.68 vs. 85.52 ± 2.84) and VLDL (16.66 ± 0.04 vs. 15.8 ± 0.11) levels were elevated while HDL (2.24 ± 0.11 vs. 6.09 ± 0.31) values was reduced in group III (diabetic untreated) compared to control animals. Animals in group IV (diabetic + *P. americana*) exhibited significant reductions ($p < 0.05$) in triglycerides, total cholesterol, LDL and VLDL values compared to diabetic untreated and control. HDL value in this treatment group (group IV) was also increased compared to diabetic untreated but comparable to controls. The triglyceride (66.21 ± 0.95 vs. 83.32 ± 0.23) and VLDL (13.24 ± 0.19 vs. 16.66 ± 0.04) values observed in the diabetic animals treated with glibenclamide (group V) were reduced ($p < 0.05$) while HDL (5.57 ± 0.13 vs. 2.24 ± 0.11) values were elevated compared to group III, respectively (Figures 2-6). Atherogenic index (AI) was significantly increased ($p < 0.05$) in group III compared to control and all other treatment groups (Table 2). Apoliporotein A-1 (Apo A-1) was reduced in groups III and IV compared to groups I and V while apolipoprotein B (Apo-B) valueincreased in group III and V compared to group I. Values for Apo-B in groups II and IV were significantly reduced ($p < 0.05$) compared to group III, the diabetic untreated group. The ratio of Apo-B to Apo-A1 (Apo-B/Apo-A1), a marker of cardiovascular risk, was reduced ($p < 0.05$) in groups II and IV compared to control and all other treatment groups (Table 2).

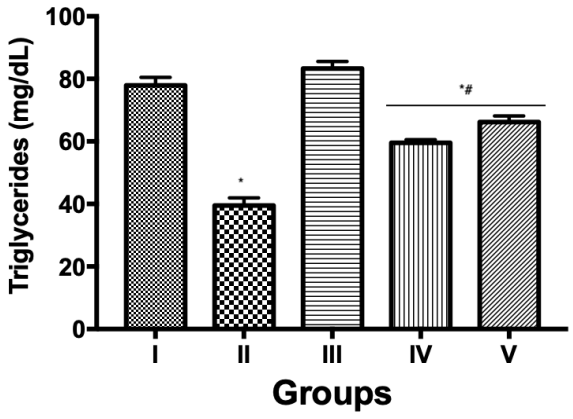

Figure 2. Triglyceride level in normal and diabetic treated groups. *Values are expressed as Mean ± SEM*; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

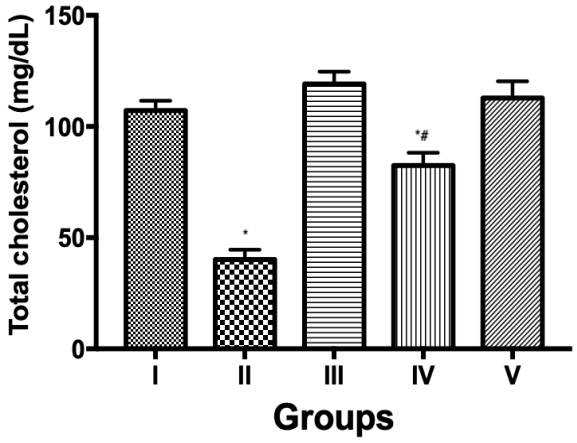

Figure 3. Total cholesterol level in normal and diabetic treated groups. *Values are expressed as Mean* ± *SEM*; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

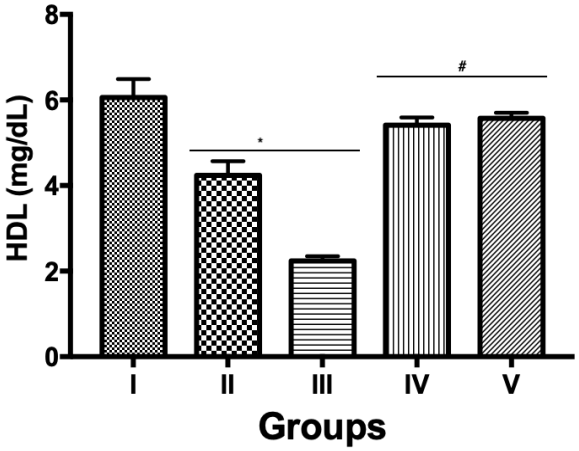

Figure 4. High Density Lipoprotein (HDL) level in normal and diabetic treated groups. *Values are expressed as Mean* ± *SEM*; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

3.4. Effect of *P. americana* on Oxidative Stress Status in Control and Experimental Groups

Superoxide dismutase (SOD) levels (μ/mL) were significantly reduced ($p < 0.05$) in group III compared to all other experimental groups. catalase (CAT) (μ/mL/min), reduced glutathione (GSH) (mM), and vitamin C (Vit-C) (mg/mL) levels were significantly reduced in groups II and III compared to all other

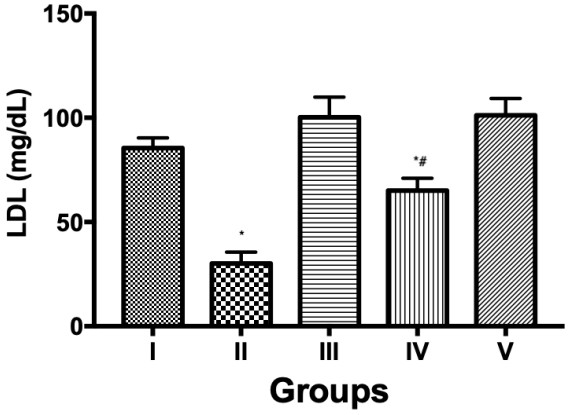

Figure 5. Low Density Lipoprotein (LDL) level in normal and diabetic treated groups. *Values are expressed as Mean ± SEM*; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

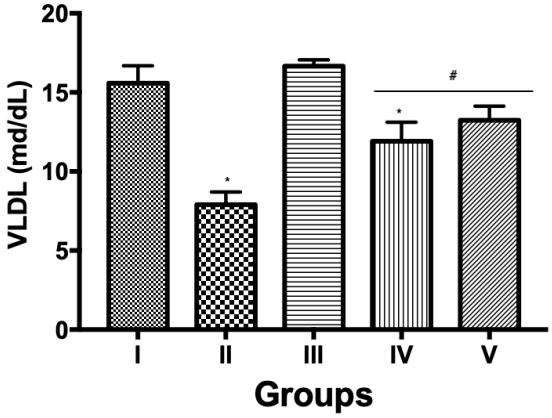

Figure 6. Very Low Density Lipoprotein (VLDL) level in normal and diabetic treated groups. *Values are expressed as Mean ± SEM*; n = 5; *indicates values that are significantly different from group 1 (control), while # indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide.

experimental groups. Malondialdehyde (MDA) (μM), a marker of lipid peroxidation, was elevated ($p < 0.05$) in group III compared to other experimental groups (Table 3).

3.5. Effects of *P. americana* on the Histology of the Heart and Pancreas

The cardiac photomicrographs obtained from animals in group I (control)

Table 2. Effect of *P. americana* on cardiovascular disease risk factors in control and diabetic treated groups.

Parameters	I	II	III	IV	V
AI	1.25 ± 0.02	1.27 ± 0.06	1.72 ± 0.01	1.18 ± 0.03	1.33 ± 0.01
Apo-A1 (mg/dl)	31.12 ± 0.32	29.76 ± 0.28	28.41 ± 0.15	29.5 ± 0.15	32.16 ± 0.39
Apo-B (mg/dl)	9.20 ± 0.25	6.79 ± 0.55	10.69 ± 0.36	7.64 ± 0.77	12.02 ± 0.28
Apo-B/Apo-1	0.32 ± 0.02	0.23 ± 0.02	0.37 ± 0.01	0.26 ± 0.03	0.38 ± 0.01

Values are expressed as Mean ± SEM; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide. AI = Atherogenic index, Apo-A1 = Apoliporotein A-1, Apo-B = Apolipoprotein B.

Table 3. Effect of *P. americana* on markers of oxidative stress in control and diabetic treated groups.

Parameters	I	II	III	IV	V
SOD (μ/mL)	0.57 ± 0.04	0.61 ± 0.02	0.23 ± 0.04	0.49 ± 0.06	0.40 ± 0.03
CAT (μ/mL/min)	27.51 ± 0.5	25.95 ± 0.23	25.3 ± 0.2	26.67 ± 0.44	28.79 ± 0.34
GSH (mM)	1.27 ± 0.09	0.81 ± 0.04^{c}	0.94 ± 0.02^{c}	1.83 ± 0.11^{c*}	1.24 ± 0.08*
Vit-C (mg/ml)	0.23 ± 0.01	0.17 ± 0.01^{c}	0.19 ± 0.00^{c}	0.2 ± 0.01	0.21 ± 0.01
MDA (μM)	5.63 ± 0.25	5.38 ± 0.23*	6.67 ± 0.07^{c}	5.57 ± 0.13*	6.04 ± 0.2*

Values are expressed as Mean ± SEM; n = 5; *indicates values that are significantly different from group 1 (control), while #indicates values that are significantly different from group III (diabetic untreated). I = Control, II = *P. americana* only, III = Diabetic untreated, IV = Diabetic + *P. americana*, V = Diabetic + Glibenclamide. SOD = Superoxide dismutase, CAT = Catalase, GSH = Reduced glutathione, Vit-C = Vitamin C, MDA = Malondialdehyde.

(**Figure 7(a)**) showed myofibres that were arranged in parallel with normal nuclei orientation. Photomicrographs from group II (**Figure 7(b)**) also showed myofibres that were arranged in parallel; however, there is also some evidence of coagulative necrosis. Cardiac photomicrographs from group III (diabetic untreated) (**Figure 7(c)**) exhibited myofibres with atrophy and patchy degeneration. In group IV (diabetic + *P. americana*) (**Figure 7(d)**), the photomicrographs displayed normal myofibres as well as a few myofibres with lost nuclei. Cardiac photomicrographs obtained from group V (diabetic + glibenclamide) (**Figure 7(e)**) showed normal myofibres and some evidence focal hyperplasia. Pancreatic photomicrographs obtained from groups I (control) (**Figure 8(a)**), II (*P. americana* only) (**Figure 8(b)**) and IV (diabetic + *P. americana*) (**Figure 8(d)**) showed no observable lesion. While the photomicrographs from the diabetic untreated group (group III) (**Figure 8(c)**) exhibited atrophy of pancreatic acini and islet

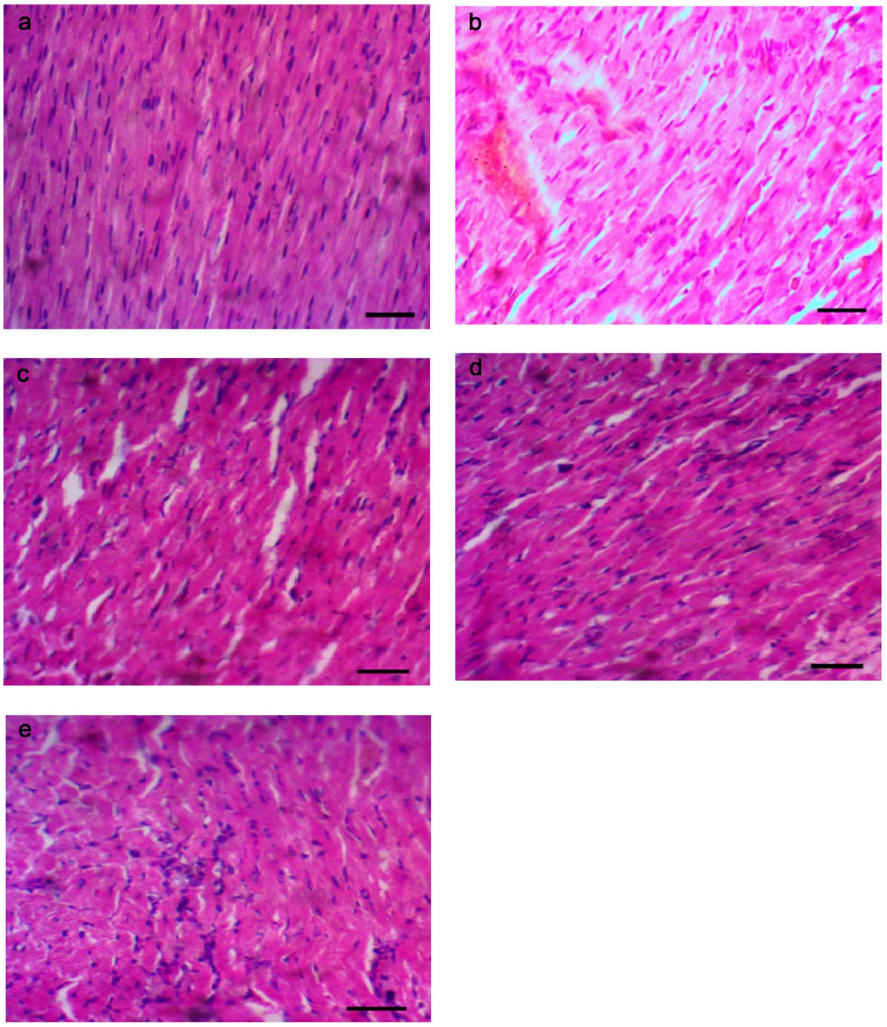

Figure 7. (a)-(e). Photomicrograph of the heart in control and diabetic treatment groups (×400, Scale bar 50 μm). Sections in the control group (a) indicate s myofibres arranged in parallel with normal oriented nuclei. No observable lesion was seen. Sections in group II (b) also show myofibres arranged in parallel, however some coagulative necrosis is observed. Group III samples (c) exhibited atrophy and patchy degeneration of the myofibres while group IV (d) shows normal myofibres as well as myofibres with lost nuclei. Sections in group V (e) normal myofibres and some evidence focal hyperplasia.

cells. Sample from the diabetic animals treated with glibenclamide (**Figure 8(e)**) exhibited moderate diffuse acinar atrophy.

4. Discussion and Conclusions

Hyperglycaemia in diabetes mellitus has been reported to result in abnormalities in lipid metabolism often referred to as diabetic dyslipidaemia with changes in lipoprotein activity that can either be quantitative (increased triglyceride and decreased HDL levels), qualitative (increased VLDL and small, dense LDLs, as well as increased triacylglycerol content of LDL and HDL, glycation of apolipoproteins and increased susceptibility of LDL to oxidation) and or kinetic (increased VLDL production, decreased VLDL catabolism and increased HDL

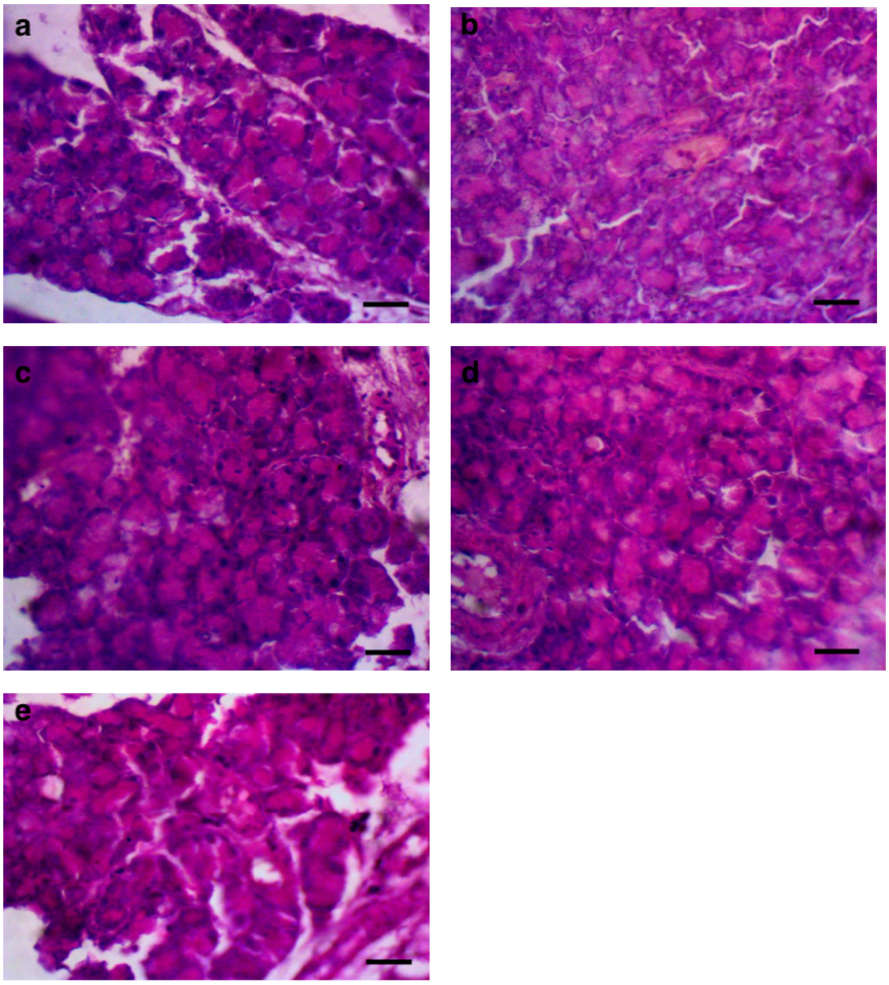

Figure 8. (a)-(e). Photomicrograph of pancreas in control and diabetic treatment groups (×400, Scale bar 50 μm). Sections from the groups I (a) and II (b) show no observable lesions while sections from group III (c) exhibited atrophy of pancreatic acini and islet cells. Group IV samples (d) showed no observable lesions while samples from group V (e) showed moderate diffuse acinar atrophy.

catabolism) [27] [28] [29]. These lipoprotein abnormalities result in a shift towards a more atherogenic lipid profile with severe consequences for the cardiovascular system.

Sustained hyperglycaemia, lipid abnormalities consistent with diabetic dyslipidaemia (increased triglyceride, total cholesterol, LDL VLDL, and reduced HDL) were observed in the diabetic untreated group in this study which is consistent with other reports using alloxan-induced diabetes experimental model to investigate lipid abnormalities in diabetes mellitus [30]. The hyperglycaemia observed in these diabetic untreated animals (group III) may be attributed to the effects of alloxan monohydrate which is known to selectively target pancreatic beta cells resulting in necrosis [17] [31] and hence result in inadequate or reduced insulin secretion which was also observed in this experimental group (**Figure 1** and **Figure 8(c)**). In addition to facilitating glucose entry in adipocytes, insulin also inhibits the breakdown of fat in adipose tissue by inhibiting

the activity of intracellular hormone sensitive lipase which hydrolyses triglycerides to release fatty acids [32]. Insulin also directly inhibits hepatic VLDL production and promotes the clearance of LDL by increasing LDL-receptor expression and activity [32]. Hence, when insulin secretion is inadequate or deficient, these aforementioned actions of insulin are impeded resulting in dyslipidaemia as observed in the diabetic untreated group in this study. The observed dyslipidaemia noted could also have resulted in micro and macrovascular complications and hence, account for the patchy degeneration and atrophy of cardiac myofibres observed in the diabetic untreated animals (group III) (**Figure 7(c)**). Also, this group exhibited a higher atherogenic index (**Table 2**), thus suggesting an increased risk for the development of cardiovascular complications in this group.

Persea americana has been reported to possess a number of phytochemicals such as alkaloids, glycosides, oxalate, phytate, phenol, saponins, steroid, and tannins which have been reported to be effective against hepato-toxicity, inflammation, cancer, hypertension amongst other disease conditions [33]. Gas Chromatography-Mass Spectrometry (GCMS) of various parts of *P. americana* has been evaluated and bioactive compounds seen have been reported to demonstrate analgesic, anti-inflammatory, antipyretic, and antihyperglycemic properties [34] [35]. Therefore, the reduction of hyperglycaemia observed in diabetic animals treated with *P. americana* which was also comparable to that obtained with glibenclamide treatment (group IV), in accordance with previous studies [12]. This hypoglycaemic activity, though not as potent as that of glibenclamide, may be ascribed to the bioactive phytocompounds in the hydro-ethanol pulp extract and the ability of the extract to stimulate regeneration of the pancreatic islet cells as observed in the histological evaluation of pancreas in this study (**Figure 8(d)**). The regenerated pancreatic islet may have also accounted for the increase in insulin secretion seen with *P. americana* treatment (group V) compared to diabetic untreated (**Figure 1**). Treatment with the extract also ameliorated diabetes induced dyslipidaemia, perhaps via increased insulin mediated lipid regulatory mechanisms [32], and impeded cardiac fibre degeneration and atrophy (**Figure 7(d)**), thereby suggesting the potentials of the hydro-ethanol extracts of *P. americana* fruit pulp in preventing diabetes mellitus related atherosclerosis and heart diseases [36]. However, *P. americana* only treated group showed decreased levels of triglycerides, total cholesterol, HDL, LDL and VLDL compared to control level which aligns with the observation of Dreher *et al.* [37] who stated that consumption of *P. americana* significantly reduces total cholesterol, low density lipoprotein cholesterol (LDL-C), triglycerides and high-density lipoprotein cholesterol (HDL-C) respectively in the blood. It is also noteworthy that though cardiac fibres seen in the *P. americana* group were normal, some level of necrosis was also seen (**Figure 7(b)**).

Apolipoprotein (Apo), a protein component of plasma lipoprotein synthesized mainly in the liver, binds to and transports blood lipids to various tissues of the body for metabolism and utilization [38]. While Apolipoprotein A1 (ApoA1)

is the primary protein component of high-density lipoprotein (HDL), Apolipoprotein B (ApoB) is the primary protein component of low-density lipoprotein (LDL) [39]. The ratio of ApoB/apoA1 has been described as an effective predictor for the risk of coronary heart disease development with increased values indicating an increased risk of atherosclerotic cardiovascular disease, independently of LDL and HDL cholesterol concentrations [39]. In this study, ApoB/A1 was significantly reduced in the *P. americana* treated diabetic group compared to diabetic untreated (**Table 2**) suggesting that the extract may mitigate the development of atherosclerosis and coronary disease often associated with diabetes mellitus. Furthermore, the atherogenic index, a novel marker for assessing the risk of atherogenicity and cardio-metabolic health, was reduced in the diabetic group treated with extract compared to diabetic untreated which reiterates the likely potential in the consumption of the hydro-ethanolic extract of *P. americana* fruit in diabetes mellitus. Interestingly, diabetic animals treated with glibenclamide also had elevated ApoB/A1 and atherogenic index compared to *P. americana* treated diabetic animals (**Table 2**). This may likely be due to the persistence of elevated triglyceride, total cholesterol and LDL despite increased HDL levels following treatment with a standard anti-diabetic agent, glibenclamide (**Figures 2-5**).

Hyperglycaemia as it occurs in diabetes mellitus, has also been reported to impair intracellular antioxidant defence mechanism resulting in over-production of free radicals by non-enzymatic glycation of serum and cellular proteins thereby rendering cells vulnerable to oxidative stress [40]. In this study, the significant alterations in oxidant-antioxidant status (increased MDA and reduced GSH, Vitamin C level, SOD and CAT activity) that occurred following diabetes were mitigated by treatment of diabetic rats with *P. americana* fruit hydro-ethanol extract. The antioxidant effect of the extract is in accordance with other studies which had also ascribed this to the presence of the bioactive compound in *P. americana* that is capable of scavenging and mopping up excessive free radicals produced during diabetes mellitus [12].

In conclusion, this study suggests that in experimental diabetes mellitus, treatment with hydro-ethanol extract of *Persea americana* fruit pulp may mitigate hyperglycaemia, oxidative stress, diabetic-induced dyslipidaemia and cardiotoxicity. The effects of the extract are mediated by the reported phytochemicals present in the plant, *Persea americana*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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