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RESEARCH ARTICLE

Vascular Effects of Avocado Seed Glycosides during Diabetes-induced Endothelial Damage

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Abstract: ***Background and Objectives*:** The relationship between vascular damage and diabetes mellitus was exploited using avocado seed extracts. The purpose of the study was to understand the therapeutic relevance of glycosides compared to standard vascular and anti-diabetic drugs. Constituent Avocado Seed Glycosides (ASG) were analysed and administered to rats with Diabetes-Induced Vascular Damage (DIVD).

***Methods*:** The rats were first administered with streptozotocin and screened after seven days for alterations in blood glucose, insulin, vascular cell adhesion molecule (VCAM-1), Von Willebrand factor (VWF), Renin-Angiotensin-Aldosterone System (RAS), eNOx, and endothelin-1 (ET-1). Only rats that satisfied these criteria were recruited and treated with either glibenclamide, met.su + losart, or 200 mg/kg body weight ASG for 28 days.

***Results*:** There was an abundance of digitoxin (13.41 mg/100g), digoxin (17.98 mg/100g), avicularin (165.85 mg/100g), and hyperoside (282.51 mg/100g). ASG or met.su + losart exhibited slight modulatory properties on glucose homeostasis. Rats with DIVD showed elevated renin, angiotensin, VCAM-1 and Lp-PLA2 levels but slightly decreased with glibenclamide treatment and normalized with ASG or met.su + losart administration. All treatments normalized Hcy levels. DIVD caused the overproduction of CnT, LDH, Crt-K, LDL-c, TG, and TC and suppressed HDL-c but was completely normalized by the ASG. Water intake remained altered in treated rats.

***Conclusion*:** The ASG had no relevant effect on glucose homeostasis during DIVD but showed significant vasoprotective properties.

Keywords: Vascular damage, endothelium, avocado seed, glycosides, cardiac integrity, diabetes.

1. INTRODUCTION

The worldwide prevalence of diabetes stretches over 100 million persons, whereby up to 95 % are incidences of non-insulin dependent (Type 2) diabetes mellitus (NIDDM) [1]. NIDDM features a two tofourfold susceptibility to cardio-vascular dysfunction attributable to the adverse effects of free radicals and hyperglycemia on the vascular system [2]. According to Creager *et al*. [1], vascular complications are the primary causes of death and disability in diabetic subjects.The complications initiate from abnormal glucose homeostasis to a continuum that eventually impairs the cardiovascular system. However, it is thought that the first incidence preceding vascular complications is the loss of endothelial functional integrity [3]. The vascular endothelium is integral to the maintenance of vascular biology and serves as a structural barrier between the lumen and vessel wall.

Endothelial dysfunction implies a condition where the endothelium can no longer effectively promote vasodilation, anti-aggregation, fibrinolysis, and other physiological effects. The endothelial cells achieve these physiological effects through the secretion of several mediators, which are either vasodilators such as endothelium-derived hyperpolarizing factor, prostacyclin, and nitric oxide, or vasoconstrictors, *e.g*., thromboxane A_2 and endothelin-1.Among these mediators secreted by the endothelium, nitric oxide (NO) is of utmost importance. The bioavailability of NO is crucial to vascular health as it regulates vasodilation, averts endogenous injury to blood vessels, as well as preventing migration and proliferation of vascular smooth muscle cells [4].

Endothelial perturbations as a function of impaired secretion of NO is well documented in clinical and experimental diabetes [5]. However, not all previous clinical studies associated type 2 diabetes with abnormal endothelium-dependent vasodilation [6, 7]. Several experimental findings support the view that endothelial NO synthesis is suppressed on account of hyperglycemia as a result of oxygen-derived free radicals [8]. In addition, endothelium-dependent vasodilation

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is suppressed during insulin resistance, a hallmark of type 2 diabetes mellitus [9]. Insulin is a stimulant of NO synthase responsible for the synthesis of NO, thereby mediating endothelium-dependent vasodilation. Furthermore, insulin sensitivity is greatly affected by endothelin-1 (ET-1), which is one of the most potent vasoconstrictors [10]. ET-1 is the endothelin's primary cardiovascular isoform involved in the aetiology of cardiovascular dysfunction. Ithas been reportedly over-secreted during type 2 diabetes mellitus. During diabetes mellitus, an alteration in ET-1 synthesis is regarded as an initial outcome rather than a late-stage occurrence of the disease [11]. Some other mediators of the vascular endothelial system affected by insulin resistance and hyperglycemia are the Von Willebrand Factor (VWF) [12], renin-angiotensin system [13], cardiac troponin [14], lipoprotein-associated phospholipase A2 [15], and vascular cell adhesion molecule [16]. Thus, the pharmacotherapy of vasoprotective drugs is established, at least in part, based on their modulatory effects on these mediators.

Many plant constituents have been identified for their potent antidiabetic effect. Among these constituents are glycosides, terpenoids, alkaloids, and flavonoids [17]. Glycosides presently recognized for their antidiabetic effects include hederagenin, jamboline, myrciacitrins, pelargonidin 3-O-α-L rhamnoside, vitexin, isovitexin, and isohamnetin [17]. The vasoprotective properties of ouabain, digoxin, and digitoxin are also well documented [18]. Avocados (*Perseaamericana*) have been used since time immemorial for nutritional and ethnomedicinal purposes [19]. The seeds are rich in phytochemicals, and have been applied for the treatment of various diseases, including diabetes [20], hypertension [21], and hypercholesterolemia [22]. Phytochemical analysis of the avocado seeds has shown various constituent phenols, flavonoids, phytosterols, and terpenoids [23]. However, a detailed characterization of constituent glycosides in the avocado seeds is yet to be carried out.This study proceeded to determine the constituent glycosides in the avocado seeds, and evaluated their effects on circulating markers of vascular and endothelial damage under streptozotocin-induced diabetes mellitus.

2. METHODOLOGY

The ripe avocado fruit (*Persea americana*) was purchased from the local markets in Owerri Imo State Nigeria and taken to the Department of Plant Science and Biotechnology, Imo State University, Owerri, Imo State, Nigeria for identification.

The seeds were harvested, pulverized in an electric grinder and sundried for 72 hrs.The seeds were further ground repeatedly into finer particles (0.05mm) before the extraction of its glycosides. The procedure described by Morsy *et al*. [24] was adopted for the extraction of the avocado seed glycosides (ASG). An amount of 100g ground seeds was immersed for 3 days in 200 ml toluene at 37°C, filtered with a Whatman 50 filter paper and transferred into 100 ml lead hydroxide for the precipitation of phenolic and tannic acids. The extract was filtered again with a Sigma Aldrich Hyflo® Super-Cel®.The pH of the extract was then adjusted to 6. A rotary evaporator under a vacuum was then used to concentrate the filtrate to 5ml. The filtrate was then subjected tofractionation,using10 ml each of ether, chloroform, and a mixture of chloroform and alcohol in a ratio of 3:2. The fractionated extractswere mixed all together and concentrated to 1ml. The extract was further analyzed for glycoside composition using gas chromatography under the following conditions; the chromatograph was an HP6890 GC powered with an HP Chemstation Rev A09.01[1206] software coupled to a flame ionization detector operating at a temperature of 320^{o}C. An AC-5 capillary column of dimension 30m x 0.25mm x 0.25µm, using nitrogen as the column gas at 28 psi and 40psi compressed air, was used. A split injection mode was applied with a ratio of 20:1 and an inlet temperature of 250^{o}C. The oven program was set at 70^{0}C initial temperature for 5 mins for 12^{0}C/min ramping for 20 min. The relative peak percentage areas shown by the detector were used to obtain the concentrations of the components while the identification of the constituents was carried out with the aid of the database of the National Institute of Science and Technology (NIST).

2.1. Experimental Design

Handling of micealigned with Principles of Laboratory Animal Care (NIH Publication No. 85-23). Ethical approval was granted by the Department of Biochemistry, Imo State University Ethics Committee (IMSU/BCM/ETS/20180619). An LD_{50} for the avocado seed glycosides was carried out on mice according to the method described by Amadi *et al*. [25] and was >400 mg/kg b.w. The environment for the experiment was maintained with a 12-hour light/ dark cycle at a moderate temperature of 24±2°C. Eighty (80) inbred male rats between 200-220 g were subjected to overnight fasting and afterwards, diabetes was induced by injecting intraperitoneally once, 60 mg/kg b.w freshly prepared streptozotocin (Sigma Aldrich) in citrate buffer (0.1M). After 7 days, blood samples were collected from the tail vein and analysed. Rats with > 250 mg/dl blood glucose and altered levels of insulin,eNOx, RAS, VCAM-1, VWF, and ET-1 were recruited as rats with diabetes-induced vascular dysfunction.

The recruited rats (60) were randomly grouped into six equal groups.The first group comprised of control non-diabetic rats only. These had been administered with rat chow and water. Group two, three and four were untreated diabetic rats, treated with 5 mg/kg b.wglibenclamide (Sigma Aldrich), and treated with 5 mg/kg b.w metoprolol succinate (met.su) and losartan (Sigma Aldrich), respectively. Group five and six were non-diabetic and diabetic rats administered with 200 mg/kg b.w ASG, respectively. All rats had free access to normal rat chow and water. The heartbeat, water intake and feed intakes were monitored daily. After 28 days, the animals were anesthetized with 2% isoflurane and 1 ml blood samples collected with disposable syringes and separated by centrifugation for parameters requiring sera. The pancreas histology was obtained after immediate excision from the anesthesized animalsand fixing with formalin -80°C.

2.2. Determination of Indicators of Glucose Homeostasis

Blood glucose was estimated using the colorimetric glucose oxidase method. Plasma insulin and glucagon were obtained following the instruction on their respective ELISA

kits (Sigma Aldrich Chemical, St. Louis, MO, USA). C-peptide was determined using rat C-peptide ELISA kit (MybiosourceSan Diego, USA).

2.3. Determination of Vascular and Endothelium Dysfunction Indicators

The renin and angiotensin activities were determined using ELISA kits (MybiosourceSan Diego, USA). The Lp-PLA2 was determined with Rat lipoprotein-associated phospholipase A2, Lp-PLA2 ELISA Kit (MybiosourceSan Diego, USA), while the VCAM-1 was assayed using ELISA assay kits (Rat VCAM-1) (MybiosourceSan Diego, USA). The eNOx was examined by colorimetric determination with NOx kit (Cayman ChemicalAnn Arbor, MI, USA) at 530 nm while the respective rat ELISA kits from MyBioSource (San Diego, USA) were used for the determination of Von Willebrand Factor (VWF), Endothelin 1 (ET-1), and Homocysteine (Hcy).

2.4. Determination of Indicators of Cardiac Integrity

Diagnostic ELISA kits (Eastbiopharm , Hangzhou, China) were used for troponin determination, while lactate dehydrogenase (LDH) colorimetric kit (Sigma Aldrich, St. Louis, MO, USA) was used for the estimation of LDH. Creatinine kinase was determined with a spectrophotometer according to the procedure illustrated by Horder *et al*. [26], while aBT-3000 auto-analyzer (Diamond Diagnostics Inc, Holliston, MA, USA) was used for the estimation of high-density lipoprotein cholesterol, triglycerides, and total cholesterol. Low density lipoproteins cholesterol (LDL-c) was obtained by formula (LDL-c = total cholesterol - high density lipoprotein cholesterol – (triglycerides / 5)).

2.5. Statistical Analysis

The data was presented as mean ± standard deviation of triplicates. The data was compared using Least Standard Deviation of statistical package for sciences and social sciences (SPSS) with one way ANOVA and considered significant at 95 % confidence interval ($p < 0.05$).

3. RESULTS

Fourteen glycosides from seeds of avocado pear were detected, as shown in Table **1**. Arbutin, linamarin and salicin were the first three isolated compounds at 17.361, 18.052, and 18.859 mins retention times. The 4^{th}, 5^{th}, and 6^{th} compounds were respectively isolated at 19.100, 19.518, 20.472 mins retention times, and identified as artemetin, amygdalin, and ouabain. Dhurrin, prunasin, digitoxin, and digoxin were identified as the 7^{th}, 8^{th}, 9^{th}, and 10^{th} isolated compounds at 21.266, 21.501, 23.362, and 23.232 minutes retention times.Lotaustralin, avicularin, hyperoside, and maysin at 23.968, 24.791, 25.867, and 26.355 mins retention times, were the last four isolated glycosides from the avocado seeds.

Under diabetic conditions, as shown in Table **2**, untreated rats produced elevated blood glucose and glucagon. The result showed that treatment with glibenclamideunder diabetic conditions produced blood glucose, and glucagon levels comparable to the normal control.Similar to metsu + losartan, ASG did not completely reverse the effect of streptozotocin on blood glucose, and glucagon, after 28 days. The production of insulin and C-peptide was suppressed following administration of streptozotocin, and treatment with either metsu + losart or ASG produced no complete modulation. We further observed that under normal

Table 1. Glycoside composition of pear seed.

Ret.Time	Area (pA*s)	Amt/Area	Amount (mg/100g)	Name
17.361	41.39084	5.44e-8	2.25e-6	Arbutin
18.052	101.11646	2.06e-10	2.08e-8	Linamarin
18.589	43.48333	1.04e-6	4.52e-5	Salicin
19.100	168.46451	3.93e-3	6.63e-1	Artemetin
19.518	107.93012	7.70e-3	8.31e-1	Amygdalin
20.472	308.83080	4.35e-6	9.09e-4	Ouabain
21.266	41.56594	5.76e-7	2.39e-5	Dhurrin
21.501	58.08210	3.40e-7	1.97e-5	Prunasin
22.362	56.23954	2.38e-1	13.41	Digitoxin
23.232	77.68143	2.31e-1	17.98	Digoxin
23.968	75.31078	5.70e-6	4.29e-4	Lotaustralin
24.791	28.65691	5.78	165.83	Avicularin
25.867	23.27950	12.13	282.51	Hyperoside
26.355	61.95982	1.32e-5	8.23e-4	Maysin

Table 2. Indicators of glucose homeostasis.

Groups	Blood Glucose (mg/dl)	Insulin (ng/ml)	Glucagon (pg/ml)	C-peptide (ng/ml)
Ctrl	82.4±4.9[a]	2.7±0.5[a]	107.1±9.2[a]	0.9±0.1[a]
Diabetic	286.6±13.8[b]	1.2±0.1[b]	159.2±7.1[b]	0.4±0.03[b]
Diab + glib	85.7±6.2[a]	2.4±0.4[a]	116.3±5.3[a]	0.8±0.07[a]
Diab + metsu + losart	201.5±11.5[c]	1.4±0.2[b]	141.9±10.4[c]	0.6±0.04[c]
ASG	79.5±2.6[a]	2.9±0.7[a]	111.6±8.2[a]	0.8±0.04[a]
Diab + ASG	189.2±8.3[d]	1.8±0.1[c]	137.4±9.0[c]	0.5±0.03[d]

Note: Values are means ± S.D of triplicates. Values bearing different superscript letter(s) (a-d) are significantly different ($p < 0.05$). Glib – glibenclamide, metsu – metoprolol succinate, losart – losartan, ASG -avocado seed glycosides.

Table 3. Circulating markers of vascular dysfunction during diabetes-induced vascular dysfunction.

Groups	Renin (ng/ml)	Angiotensin (pg/ml)	VCAM-1 (ng/ml)	Lp-PLA2 (ng/ml)
Ctrl	51.3±3.8[ae]	82.4±2.5[a]	45.9±2.2[a]	50.9±2.4[ae]
Diabetic	73.8±4.6[b]	109.6±3.9[b]	63.1±2.8[b]	116.2±3.7[b]
Diabetic + glib	65.3±2.9[c]	96.4±4.1[c]	53.6±3.2[c]	85.8±3.9[c]
Diabetic + metsu+losart	49.1±3.3[ad]	84.2±3.4[a]	42.9±2.5[ad]	53.1±2.9[a]
ASG	45.7±2.7[d]	76.6±2.6[d]	40.8±2.8[d]	46.6±2.1[e]
Diab+ASG	54.7±2.1[e]	81.1±3.7[a]	43.4±2.1[ad]	55.2±3.2[a]

Note: Values are means ± S.D of triplicates. Values bearing different superscript letter(s) (a-e) are significantly different ($p < 0.05$). Glib – glibenclamide, metsu – metoprolol succinate, losart – losartan, ASG – avocado seed glycosides.

Table 4. Circulating markers of endothelial integrity during diabetes-induced vascular dysfunction.

Groups	VWF (ng/ml)	eNOx (µM/L)	Endoth (pg/ml)	Homocysteine (µM/L)
Ctrl	26.8±1.7[a]	32.5±3.3[a]	16.2±1.1[ad]	48.2±2.9[ac]
Diabetic	41.9±2.6[b]	20.6±1.4[b]	29.3±1.9[b]	57.8±3.4[b]
Diabetic + glib	35.0±1.9[c]	26.4±1.7[c]	24.7±2.7[c]	50.9±2.1[a]
Diabetic + metsu+losart	25.3±2.2[a]	30.7±2.8[a]	18.6±2.3[a]	47.3±2.6[ac]
ASG	27.5±2.1[a]	33.9±2.0[a]	14.3±2.1[d]	45.8±2.4[c]
Diabetic +ASG	30.6±3.8[a]	29.4±1.5[ac]	17.4±2.4[ad]	49.0±2.2[a]

Note: Values are means ± S.D of triplicates. Values bearing different superscript letter(s) (a-d) are significantly different ($p < 0.05$). Glib – glibenclamide, metsu – metoprolol succinate, losart – losartan, ASG – avocado seed glycosides.

conditions, ASG does not alter any of blood glucose, insulin, glucagon, or C-peptide levels.

The activities of renin and angiotensin, vascular cell adhesion molecule 1 (VCAM-1), and lipopolysaccharide phospholipase A2 levels during DIVD and consequent treatment with eitherglibenclamide, metsu + losart, and ASG are shown in Table **3**. Under normal conditions, the ASG produced significantly lower renin and angiotensin levels than the control. The plasma renin activities and angiotensin levels after 28 days of DIVD remained higher than those of the control group while treatment with glibenclamide produced a slight lowering effect. The results showed that the application of ASG or metsu + losart under DIVD normalized the plasma renin and angiotensin levels. Both VCAM-1 and Lp-PLA2 were significantly elevated during DIVD, and remained elevated after 28 days. Glibenclamide treatment produced a slight lowering effect on the VCAM-1 and Lp-PLA2 levels, whereas treatment using either metsu + losart or ASG produced equivalent levels to those of the control group.

Further assessment of the activities of the Von Willebrand Factor, endothelial nitric oxide, endothelin, and

Table 5. Cardiac integrity during diabetes-induced vascular dysfunction.

Groups	CnT (ng/ml)	LDH (U/L)	Crt-K	TC	HDL	TG	LDL
Ctrl	5.3±0.6[a]	175.8±8.1[ae]	365.5±7.1[ae]	67.0±3.1[a]	35.4±1.0[a]	50.6±2.7[a]	21.4±3.6[a]
Diabetic	6.7±0.4[b]	272.1±7.3[b]	477.4±12.3[b]	83.7±3.8[b]	19.8±1.7[b]	71.6±3.6[b]	49.5±2.7[b]
Diabetic + glib	5.9±0.4[a]	233.8±9.3[c]	426.6±10.1[c]	75.8±1.9[c]	32.8±3.3[a]	57.1±1.6[c]	31.6±5.6[c]
Diabetic + metsu+losart	5.2±0.5[a]	187.4±5.0[d]	388.0±10.9[d]	69.4±3.1[a]	33.8±1.5[a]	48.3±3.1[a]	25.9±4.0[a]
ASG	5.0±0.7[a]	168.1±8.4[a]	352.4±9.3[a]	67.7±1.9[a]	35.0±1.8[a]	51.7±3.3[a]	22.4±4.4[a]
Diabetic + ASG	5.6±0.3[a]	180.8±6.5[e]	371.5±8.2[e]	65.9±4.1[a]	33.1±2.1[a]	50.8±3.5[a]	22.6±5.5[a]

Note: Values are means ± S.D of triplicates. Values bearing different superscript letter(s) (a-d) are significantly different ($p < 0.05$). Glib – glibenclamide, metsu – metoprolol succinate, losart – losartan, ASG – avocado seed glycosides.

homocysteine during normoglycemic and hyperglycaemic conditions with treatment using glibenclamide, metsu + losart, or ASG is presented in Table **4**. The study showed that consequent to DIVD, the VWF, endothelin, and homocysteine remained over-secreted. The streptozotocin administration also caused the suppression of eNOx synthesis. During DIVD, the reversal of the VWF, eNOx, and endothelin alterations was not achieved using the standard antidiabetic agent, glibenclamide, whereas treatment using metsu + losart or ASG during DIVD, produced equivalent VWF, eNOx, endothelin, and homocysteine levels to those of control rats. The results also showed that the ASG under normoglycemic conditions produced no alterations on the examined markers of endothelial dysfunction.

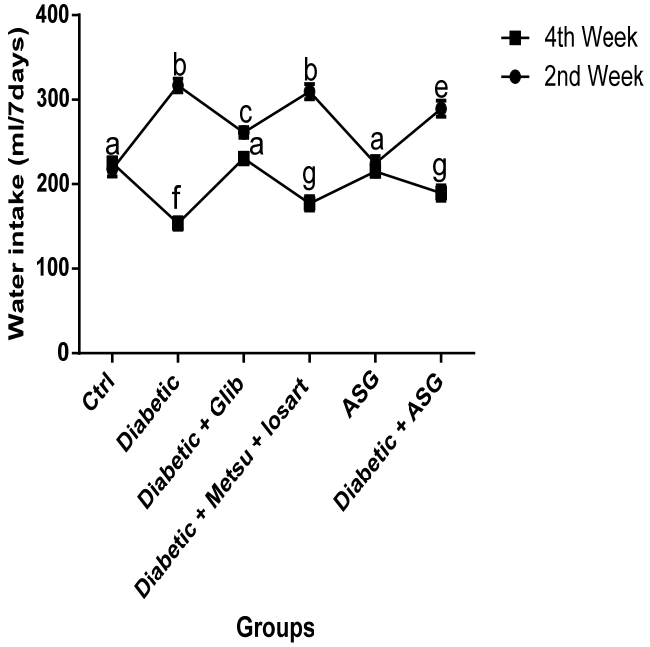

Fig. (1). Water intake levels. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

In Table **5**, after treatment with glibenclamide, metsu + losart, or ASG, some circulating markers of cardiac integrity, cardiac troponin, lactate dehydrogenase, and creatinine kinase and the lipid profile were examined during DIVD.The results showed elevated CnT, Crt-k, and LDH levels in untreated DIVD animals. Treatment using glibenclamide during DIVD, produced equivalent CnT levels to control, metsu+losart treated or ASG treated rats but was ineffective in the restoration of crt-k and LDH. Treatment of DIVD using metsu+losart significantly lowered the LDH and crt-k levels, but only ASG treatment produced equivalent levels to the normal rats. The result for the lipid profile showed elevations in TC, TG and LDL levels after 28 days of untreated DIVD. Treatment with glibenclamide only normalized the HDL levels, whereas the metsu + losart and ASG completely reversed the altered lipid profile.

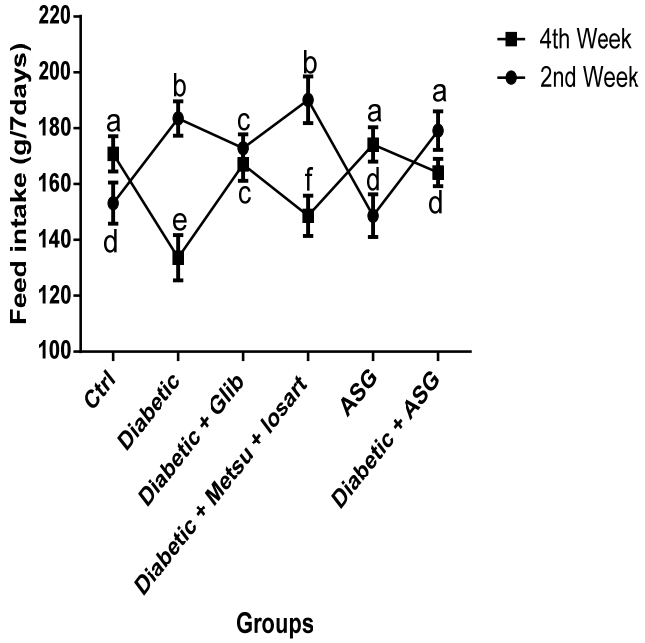

Fig. (2). Feed intake. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

Water and feed intake per 7days after DIVDfollowed by treatment with either glibenclamide, metsu + losart or the ASG, untreated rats and rats treated withlosart + met.su after two weeks showed significantly higher water and feed intake

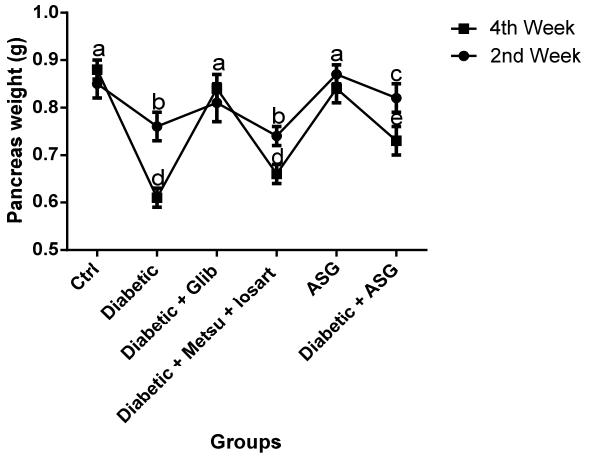

Fig. (3). Pancreas weight. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

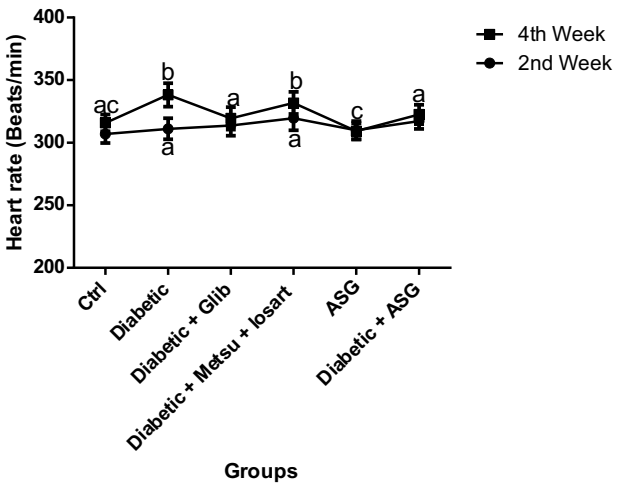

Fig. (4). Heart beat. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

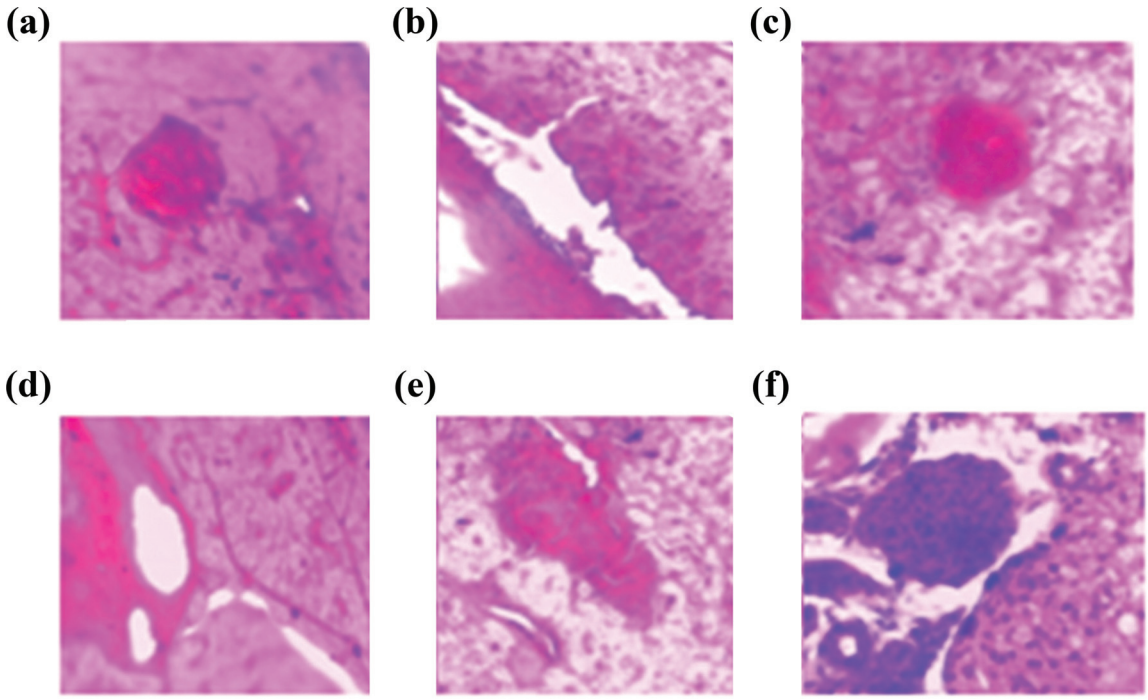

Fig. (5). a: Histologic slide from the pancreas of control rats showing intact islet cell mass. **b:** Histologic slide from the pancreas of group 2 rats showing extensively reduced islet cell mass. **c:** Histologic slide from the pancreas of group 3 rats showing intact islet cell mass. **d:** Histologic slide from the pancreas of group 4 rats showing recovering islet cell mass. **e:** Histologic slide from the pancreas of group 5 rats showing intact islet cell mass. **f:** Histologic slide from the pancreas of group 6 rats showing recovering islet cell mass. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

(Figs. **1 & 2**). Treatment with glibenclamide and ASG significantly lowered the water intake levels as compared with untreated rats, but not equivalent to the water consumption rate of normal rats. After 4 weeks, only glibenclamide proved effective in normalizing the alterations in water consumption capacities of the rats with DIVD. At both the 2 and 4 weeks, treatment of DIVD normalized the feed intake amounts. The results showed asignificant decrease in pancreas weight after the 2nd and 4th weeks following DIVD, but was normalized with glibenclamide treatment. None of the metsu + losart or ASG treatments reversed the loss of pancreas weight during DIVD. The heartbeat of the untreated rats was slightly elevated during DIVD, but normalized after four weeks. Treatment with metsu + losart and ASG after 2 weeks reversed the altered heartbeat while we found that after 4 weeks, DIVD and treatments with glibenclamide, metsu + losart or ASG produced no effects on the heartbeat. Moreover, under normoglycemic conditions, ASG was shown not to obstruct feed and water intake, heartbeat, and pancreas weight (Figs. **3, 4 & 5**).

4. DISCUSSION

The chromatographic detection of glycosides from avocado seeds yielded minute amounts of arbutin, linamarin, salicin, ouabain, dhurrin, prunasin, lotaustralin, and maysin, which make their contribution to the overall medicinal properties of the avocado seeds unlikely. However, we observed that the avocado seeds produced abundant amounts of artemetin, amygdalin, digitoxin, digoxin, avicularin, and hyperoside. Thus, the therapeutic effects of the avocado seed glycosides could initiate from these predominant glycosides. Notwithstanding the paucity of information on the medicinal applications of artemetin, it has found applications in amelioration of endothelium *via* its modulatory effects of nitric oxide synthesis [27], anti-edematogenic [28], anti-inflammatory, and free radical scavenging properties [29, 30]. Though its vascular effects have been identified [31], no report as of date suggests possible anti-diabetic effects. Amygdalin, another glycoside found in elevated quantity in the avocado seeds, has a report suggesting possible anti-diabetic effect. Further angiogenic and antitumor effects of amygdalin have been reported elsewhere [32, 33]. Similar to artemetin, digitoxin has no reported antidiabetic effects, rather, numerous studies have pointed out potent cardio-protective and anticancer potentials [33, 34]. Studies on digoxin suggest a possible ameliorative effect of glucose intolerance during type II diabetes mellitus as well as attenuates hypoglycemia [35, 36], in addition to cardiovascular effects [37, 38]. In addition, avicularin has been associated with potent antidiabetic and vascular effects [39, 40] while hyperoside has been applied for reversal of endothelial dysfunction and hypoglycemia [41, 42]. With the obvious lack of low reportage of any antidiabetic potency of these predominant glycosides, our study supports the proposition that these glycosides may be more suitable for reversal of dysfunctional vascular system and endothelium rather than any anti-diabetic effects. Excessive glucose secretion has been the hallmark of Type 2 diabetes mellitus which features suppressed insulin activities following altered β-cell integrity and insulin resistance. In our study, neither metsu + losartan nor ASG was as effective as glibenclamide in normalizing blood glucose levels after streptozotocin-induced hyperglycemia and vascular damage. This may be related to the difference in mechanisms of actions of the compounds. Glibenclamide is insulinogenic through cellular depolarization and as well produces extra-pancreatic effects [43] whereas metoprolol succinate and losartan both widely recognised drugs with vascular effects, have no effect on insulin sensitivity [44, 45]. This observation correlates with our present findings during diabetes, showing no change in insulin levels after treatment with met.su + losart. However, we envisage that during diabetic conditions, the ASG, having induced elevated insulin secretion, produces slight insulinogenic effects more than the standard vascular drugs. There are experimental suggestions supporting excessive glucagon production during diabetes [46], which are consequently normalized on applications of glucagon-suppressing agents like glibenclaminde [47]. Our results showed that the standard vascular drug has no effect on the glucagon levels during DIVD. Furthermore, we found glibenclamide potent enough to reverse alterations in C-peptide and Hb1Ac levels during DIVD. Treatment using losart + metsu produced significant modulatory properties on C-peptide and Hb1Ac, which corresponds with similar reports byJin and Pan, [48]. In the proinsulin molecule, C-peptide connects insulin A and B chain and is of immense use in clinical testing for diabetes. As C-peptide correlates with the intrinsic potentials of cells to produce insulin, our results thus indicate that the ASG and vascular drugs, during diabetes, potentiate the synthesis of insulin by the pancreas without a complete restoration.

Macrovascular complications during diabetes occur as a sequence of numerous processesthat proceed from atherosclerosis and thrombosis and by relating diabetes with cardiovascular diseases such as hypertension and atherosclerosis implicate the role of the renin-angiotensin system (RAS) in the onset and diseaseprogression. The stimulation of RAS is thus regarded as the main etiologic event in the initiation and progression of vascular damage in diabetic patients [49]. This view aligns with our findings showing prolonged activated renin and angiotensin levels following the administration of streptozotocin. Numerous clinical trials have upheld the finding that RAS blockers regress vascular complications in diabetic subjects, which explain why metsu + losart in this study completely normalized the RAS compared to slight modulation by glibenclamide, a standard anti-diabetic drug. Furthermore, this clearly elucidates the mechanism of action of the ASG patterns towards the standard vascular drug; metsu+losart rather than glibenclamide, the standard antidiabetic agent [50, 51].

Studies elsewhere have established that cellular adhesion moleculescould serve as reliableindicators of vascular dysfunctionduring diabetic conditions [52, 53]. In line with similar reports elsewhere [54, 55], our study showed significant alterations in VCAM-1 levels during DIVD, while the mild modulatory effect of glibenclamide corroborates with the reports of Papanas *et al*., [56]. As we observed, treatment of diabetes-induced vascular dysfunction with angiotensin receptor blockers portends greater potentials to normalize adhesion molecules compared to sulphonylureas such as glibenclamide. Song *et al*. [57] suggested that angiotensin receptor blockers such as telmisartan, but not losartan, modulate hyperglycemia-induced dysfunctional VCAM-1 expression by suppressing the phosphorylation of NF-κB, p65-Ser(536) and expression of IκB kinase β. Since the ASG produced an equivalent modulatory effect on the VCAM-1 levels during DIVD, we propose that the mechanism of the predominant glycosides in the ASG may occur by blockade of angiotensin receptors. Lp-PLA2 activities positively correlate with increased proneness to atherosclerosis and other cardiovascular diseases. The elevated Lp-PLA2 activities we observed during DIVD is supported by the reports of Nelson *et al*. [58] and Jackisch *et al*. [59]. However, in contrast to our findings, Basu *et al*., [60] reported that potent sulphynylureas lacked any effect on Lp-PLA2 activities during diabetes. Also, the complete restoration of Lp-PLA2 by the metsu+losart may have resulted due to the combination of angiotensin receptor blockers with inhibitors of β1 adrenergic receptors as Rizos *et al*. [61] reported that Eprosartan, an angiotensin receptor blocker, had no effect on the Lp-PLA2 activities during vascular damage. Thus, this could imply that the predominant glycosides in the avocado

seeds could achieve vascular restoration following several mechanisms in synergy.

According to HadiandJassim [62], established experimental and clinical views, the endothelial perturbations arise from both diabetes and insulin resistance which undermines the vasoprotective roles of the vascular endothelium. More so, various degrees of disruptions in circulating markers of endothelial dysfunction have been identified in streptozotocin-induced-diabetes-associated vascular damage [63]. In this study, steady elevations in VWF, eNOx, endothelin, and homocysteine after streptozotocin administration after 28 days are confirmatory of diabetes-induced endothelial dysfunction [64-66]. In our study, we found that treatment using glibenclamide slightly inhibited the VWF production after vascular damage, which contradicts the reports of Yngen *et al*., [67]. Few or no reports have been found evaluating the effect of losartan or other standard vascular drugs on VWF activities during diabetes-induced vascular disease. However, in our study, we observed that similar to the combined effect of losartan and metoprolol succinate, the ASG normalized the VWF activities after diabetes-induced alterations suggesting that these predominant glycosides potentiate endothelium repair. The maintenance of eNOx activities is particularly crucial during diabetic conditions due to its central role in stabilizing the vascular tone. The perceived hyperglycemia-induced NOx alterations are consequent to influence on protein kinase-c activities [68], which are modulated with glibenclamide treatment [69]. Another study noted that glibenclamide stimulates the endothelium by potentiating nitric oxide release [70], however, we establish from our study, the preference of standard vascular drugs for complete restoration of eNOx production during DIVD, rather than antidiabetic agents. Endothelin peptides are widely distributed in numerous tissues and perform various vascular functions. Hence, endothelin antagonists are agents of recent research interest to prevent vascular damage during diabetes. We observed that the ASG treatment inhibited endothelin similar vascular damaged rats treated with metsu + losart. Though the mechanism behind the suppression of endothelin-1 production by both the standard drug and ASG has not been fully investigated, we relate the ASG activities to those of standard endothelin receptor blockers. Yegnanarayan *et al*. [71] reported the relevance of sulfonylureas in the treatment of type 2 diabetic subjects with hyperhomocysteinemia. In our study, we confirm that the antidiabetic agent, glibenclamide, completely normalized the diabetes-induced hyperhomocysteinemia. Similarly, diabetic rats that received metsu+losart or ASG also showed a complete reversal of diabetes-induced elevated homocysteine levels. In contrast to our findings, Zhu *et al*. [72] found no correlation between losartan administration and homocysteine activities while Li *et al*. [73] demonstrated that supplementation of telmisartan abrogated hyper-homocysteinemia-induced vascular damages.

Established interrelationships exist between lipoprotein abnormalities and type 2 diabetes mellitus [74]. Characteristic dyslipidemia during diabetes, features suppressed HDL-c, predominance of triglycerides and LDL-c [75], which are predictive indicators of cardiovascular diseases. We found glibenclamide as a mild cardiomodulatory agent during DIVD, which corroborates with the reports of Derosa *et al*. (2011), and the complete restoration of the HDL-c agrees with the reports of Mughal *et al*. [76]. Shad *et al*. [77] suggested that losartan alone produced insignificant effects on lipoproteins meaning that the normalized lipoprotein levels in this study resulting from the combinatorial administration of losartan and metoprolol succinate were mostly due to metoprolol succinate. Our study showed that the ASG produced cardiomodulatory properties during diabetic conditions comparable to the effect of combinatorial treatment with losartan and metoprolol succinate, implying that the predominant glycosides in avocado seed could possess β_1 receptor blocker type properties. This is validated by the predominance of digoxin in the ASG, which is well documented for triglyceride, total cholesterol, and LDL reductions [78]. The cardiac troponin has been identified to be of substantial utility in the prediction of cardiovascular risks during diabetes mellitus [79-81] and for this reason, recent pharmaceutical interventions on cardiovascular diseases, target the troponin activities. Losartan has a potent lowering effect on troponin [82], whereas metoprolol succinate portends no change in CnT rise [83]. With this, troponin suppression during DIVD progressed *via* blockade of angiotensin receptors and could be a similar mechanism behind the restoration of normal troponin activities by the ASG. Lactate dehydrogenase and creatinine kinase are always elevated in streptozotocin-induced diabetic rats [84, 85] and mildly modulated by glibenclamide [86, 87]. Shi *et al*. [88] and Dianat *et al*. [89] showed the effectiveness of losartan in decreasing creatine kinase and LDH levels during cardiovascular dysfunction. Digoxin under mocarditis, significantly decreases LDH levels [90], whereas Kim *et al*. [91] demonstrated that avicularin significantly suppresses LDH release from cardiac cells, while Li *et al*. [92] reported that administration of hyperosides after cardiovascular disorder attenuates leakages of creatine kinase and LDH from cardiac tissues. We thus propose that our observed modulatory effect of ASG on LDH and CK during DIVD, relates to the predominance of hyperoside, avicularin and digoxin.

CONCLUSION

The avocado seed glycosides under normoglycemic conditions showed mildly negative vasoactive effects. Under diabetic induced vascular damage, the glycosides in avocado seeds or the combinatorial administration of the standard vascular drugs were ineffective in normalizing glucose homeostasis as compared to standard hypoglycaemic agents. Reversal of vascular damage during diabetic conditions was achieved with the ASG and showed equivalent potency with combinatorial administration of metoprolol succinate and losartan. We propose that thevasoprotective effect of the ASG is attributable to the synergistic effects of these predominant glycosides.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Ethical approval was granted by the Department of Biochemistry, Imo State University Ethics Committee, Nigeria (Approval No: IMSU/BCM/ETS/20180619).

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal research procedures followed were in accordance with the Principles of Laboratory Animal Care (NIH Publication No. 85-23).

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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