

**EVALUATION OF THE ANTI-DIABETIC AND BIOCHEMICAL EFFECTS OF
PHYTOCHEMICALLY CHARACTERIZED ETHANOL SEED EXTRACT OF *PERSEA
AMERICANA* (AVOCADO) ON ALLOXAN INDUCED DIABETIC RATS****Nwankpa Promise, Nwabueze Obinna Stephen and Uche Mercylyn Ezinne***

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ABSTRACT

Persea americana has been used in folklore medicine for the management of obesity, hypertension and diabetes mellitus. This research investigated the antidiabetic and biochemical effects of phytochemical characterized ethanol seed extract of *Persea americana* (ESEPA). The phytochemical characterization of seed extract of *P. americana* (SEPA) was carried out using Gas Chromatography Mass Spectrometry (GC-MS). In the acute toxicity study, animals were given oral doses of ESEPA ranging from 0.5g/kg to 5g/kg. Diabetes was induced using a single intraperitoneal injection of 150mg/kg of alloxan. For the subacute toxicity test which lasted for 14 days, rats were divided into six (6) groups of five animals each. Group 1 was normal control, group 2 negative control, group 3 positive control, groups 4,5 and 6 were given 200mg/kg, 400mg/kg and 600mg/kg respectively. Group 2-6 rats were all induced with diabetes. Standard analytical protocols were followed during the experiment. GC-MS analysis revealed 13 bioactive compounds. Animals did not show any signs of toxicity and no death was recorded after administering upto 5g/kg of EPESA to rats. Oral administration of ESEPA resulted in a significant decrease ($p<0.05$) in the level of blood glucose, packed cell volume, haemoglobin, urea, creatine, aspartate transaminase, alanine transamine, total cholesterol, triacylglycerol, low density lipoprotein when compared with negative control groups. However, plasma levels of albumin, globulin and high density lipoprotein significantly increased ($p<0.05$) when treated groups are compared with the control. This study showed ESEPA is non-toxic but contains bioactive phytochemical with pharmacological activities such as antidiabetic, antilipidemic, hepatoprotective.

KEYWORDS: *Persea americana*, diabetes mellitus, triacylglycerol, hypolipidaemia, toxicity.**INTRODUCTION**

Diabetes mellitus which is an array of signs and symptoms resulting from defective metabolism of carbohydrates, lipids and proteins is a universal health challenge with the number of individuals living with the disease rising from 200 million to 830 million between 1990 and 2022.^[1] The prevalence of diabetes has risen more rapidly in the second and third world countries and resulted in the death of 2 million people in 2021.^[2] In diabetes, the β -cells of the islets of Langerhans either fails to produce insulin, a peptide hormone that regulates glucose level in the body or the cells are insensitive to insulin resulting in a series of metabolic disorders.^[3,4] The defective metabolism of carbohydrate, lipid and protein seen in diabetes mellitus results in hyperglycaemia which remains the cardinal feature of diabetes. Complications of diabetes include nephropathy, retinopathy, vasculopathy, diabetic foot ulcer and gangrene, diabetic ketoacidosis, hyperosmolar non-ketotic coma and obstetric complications while polydipsia, polyuria, nocturia, weight loss and recurrent

immunosuppression predisposing to opportunistic infections such as candidiasis are some of the clinical features of diabetes.^[5,6] These litany of clinical features and complications of diabetes mellitus according to Ojo et al.^[5] accounts for over 1 million deaths annually worldwide. It is generally classified based on the pathogenesis and age group affected into type 1 diabetes mellitus (juvenile onset diabetes mellitus or insulin dependent diabetes mellitus) and type 2 diabetes mellitus (adult onset diabetes mellitus or non-insulin dependent diabetes). It is estimated that 7079 per 100,000 persons will suffer from diabetes by 2030.^[7]

Globally, phytomedicine has recently received great attention because of its unquantifiable contribution to the treatment of various ailments afflicting mankind especially among folks who cannot access or afford expensive orthodox medications.^[8,9,10] Bioactive compounds and metabolites present in the various plant parts have been previously shown by various researchers to be potent in the cure for diseases that affect the eyes,

liver, kidneys, heart, brain and libido.^[11,12] Medicinal plants such as *Artemisia pallens*, *Bidew Pilosa*, *Bixaoreltana*, *Teramnus labialis*, *Ageratum conyzoides*, *Magniferaindica* and *Musa sapientum* have all been shown through researches to provide credible non-orthodox approach in the treatment of diabetes mellitus.^[13,14,15] *Persea Americana* ranks among the numerous plants rich in bioactive compounds that are used to manage various ailments.

In the Nigerian health system, most patients with diabetes rely on orthodox antidiabetic medications to treat their ailment. While persons with type 1 diabetes rely on insulin injections for their well-being, patients with type 2 diabetes use biguanides especially metformin and sulphunylureas especially glibenclamide and then short acting, intermediate acting and long acting insulin injectable when hyperglycaemia remains recalcitrant or during complications, surgery and pregnancy. Life threatening adverse effects such as lactic acidosis usually seen in subjects that take biguanides, hypoglycaemia, weight gain, abdominal pain, nausea and vomiting, bloating and tolerance seen in chronic use of these antidiabetics have resulted in frantic search for alternative hypoglycaemic agents.^[15,16] Recently and as a matter of necessity, people have started searching for plant materials that are cheap, affordable and are readily available replacements with little or no side effects to manage their diabetes.^[9] *Persea Americana* seed is one of such alternatives since it contains flavonoids, saponin and other phytochemicals which are proven to possess antidiabetic capabilities.

Persea Americana tree originated from Southern Mexico but is found in most homes and farms in Southern Nigeria with a fruit that is creamy, edible and smooth. It is called avocado in English or “ubebekee” in Igbo Language Nigeria and is a flowering plant which belongs to the family Lauraceae. It is used in folklore medicine to treat diabetes, alopecia, dysentery, intestinal worms, cancer and wound healing.^[15,17,18] The seeds, fruits and leaves of *P. Americana* is rich in vitamins, micronutrients, antioxidants and other bioactive compounds.^[19] According to a study by Kooti et al.^[20] *Persea americana* also contains flavonoids, glycosides, alkaloids and terpenoids which exert hypoglycaemic effects by improving the activities of the pancreatic tissues. The high bioactive constituent of avocado and the apparent side effects and high cost of procuring the conventional tablets and injectable antidiabetic drugs have pushed diabetic patients towards embracing a cheaper and readily available plant-based remedy with wide margin of safety. This formed the basis of this research work to evaluate the phytoconstituents and toxicity profile of *P. Americana* as well as study the antidiabetic effects of this plant extract on alloxan induced wistar rats.

MATERIALS AND METHODS

Collection and identification of plant materials

Avocado pear fruit was harvested from a farmland in Okohia, Ideato South Local Government Area, Imo State, Nigeria and was taken to the department of Forestry, Michael Okpara University of Agriculture, Abia State Nigeria where it was identified by a taxonomist in the department.

Preparation of *Persea americana* seed extract

The fruit flesh was carefully removed And the seed minced into tiny particles using a grater and then oven dried at 40°C until a fixed weight was obtained. The dried particles were ground into fine powder using a locally manufactured grinding machine. Ethanol extraction was done by mixing 100g of the avocado pear powder with 1000ml of 98% ethanol and allowed to stand for three days with constant stirring using a glass rod. The resultant mixture was filtered using a Whatmann filter paper No 1 to obtain clear filtrate which was evaporated in water bath at 40°C. Eventually, the powdered extract was stored at 4°C awaiting use for laboratory analysis.

Gas Chromatography Mass Spectrometry (GC-MS) analysis of *P.americana* seed

The *Persea americana* seed extract was analysed using GC-MS. The analysis was carried out using Agilent 7890A-5975C GC-MS system. A HP5-column (30m x 0.25mm x 0.25µm), operating in electron impact mode at 70eV was utilized. The carrier gas was ultra-pure helium at a flow rate of 1.0 mL/min and a linear velocity of 37cm/s. The injector temperature was set at 250°C. The initial oven temperature was at 110°C which was programmed to increase to 280°C at the rate of 10°C/min with a hold time of 7 minutes at each increment. Injections of 0.5 µL were made in the splitless mode with a split ratio of 10: 1. The mass spectrophotometer was operated in the electron ionization mode at 70 eV. The bioactive compounds were identified by direct comparison of the retention times and mass spectral data and fragmentation pattern with those in the National Institute of Standards and Technology (NIST) library.

Animal Experiment

Thirty healthy albino rats with weight between 140-200g were bought from the laboratory animal production section of the department of Biochemistry, Abia State University, Uturu. They were kept in metal cages in a photoperiod cycle of 12h:12h (light and dark), at a room temperature of 28°C and fed with standard rat chow and clean drinking water for a period of seven days during which they were allowed to acclimatize. Internationally accepted standards for the use of animals for research were observed throughout the duration of the experiment. Ethical approval for this research was granted by Faculty of Basic Medical Sciences, Imo State University ethics committee.

Acute Toxicity Test

Acute toxicity studies were carried out using the methodology described by OECD guideline 243.^[21] Research animals (n=3/group) received a singular oral dosage of ESEPA (0.5g/kg, 1g/kg, 2g/kg, 3g/kg, 4g/kg and 5g/kg) after experimental animals were fasted for 4 hours following which the animals were observed for toxicity signs or death over a 24 hour period and then an additional 14-day observation period.

Induction of Diabetes/Blood Glucose Determination

Experimental rats received a single injection of 150mg/kg body weight of alloxan monohydrate via the peritoneal route following the determination of a baseline glucose level check in the rats. Diabetes was assumed to have been induced when the rats fasting blood sugar was greater than 120mg/dl using an Accu-check glucometer manufactured by Roche Diabetes Care Limited, United Kingdom. Following the induction of diabetes, blood glucose level was subsequently determined on weekly basis during the experiment.

Subacute Toxicity Study

The animals used for this research were split into six groups of four rats per group. Except group 1 animals that served as normal control and received water and rat chow, groups 2-6 were induced with diabetes. Group 2 alloxan induced diabetic rats received water and rat chow and served as negative control. Group 3 diabetic animals received 50mg/kg of Glucophage while groups four, five and six diabetic rats received 200mg/kg, 400mg/kg and 600mg/kg of ESEPA respectively for 14 days via oral gavage. After the 14-day ESEPA treatment, animals were fasted overnight and were sacrificed by dislocation of the cervical bones. Blood samples obtained by puncturing the cardiac muscles were introduced into EDTA containers and plain bottles for haematology and clinical chemistry analysis respectively. Body weight of rats were determined on days 0, 7 and 14 of the experiment.

Blood Analysis

Packed cell volume (PCV), haemoglobin (Hb) level, red blood cell (RBC) indices, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet level and white blood cell (WBC) counts were determined using an automated hematology analyzer

(BC-2300 model, Mindal Medical Co. China) using guidelines provided by the manufacturers.

Biochemical Analysis

Liver function tests (ALT, AST, ALP, albumin, bilirubin, globulin and total protein), kidney profile (serum urea, creatinine and electrolytes: Na⁺, K⁺, HCO₃⁻ and Cl⁻) and lipid profile (triacylglycerol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol, high density lipoprotein cholesterol and total cholesterol) were determined using standard laboratory tools made available by Randox laboratory ltd co, Antrim, United Kingdom.

Histopathology Studies

Pancreas, liver and kidneys harvested from rats were immediately introduced in a specimen bottle filled with 10% formalin. Already established standard methods were used to process and histopathologically analyze the tissue samples. This involved slicing the tissue sections, processing and staining them with haematoxylin and eosin following which the sections were studied under a German made Trinocular Carl-Zeiss microscope at 400x magnification.

Data Analysis

Microsoft excel and R-statistics version 4.3.3 were used to perform the analysis of the data obtained in this research. Results data were displayed as the mean \pm standard deviation (SD). Group comparison was performed using the analysis of variance (ANOVA) test and Tukey's post hoc test with p-value of less than 0.05 considered statistically significant.

RESULTS

GCMS Analysis

Gas Chromatography Mass Spectrometry analysis of methanol seed extract of *P.americana* is shown in table 1 with (R*, R*)-5-hydroxy-4-methyl-3-heptanone having the highest percentage composition (22.82) and 1-octenyl succinic anhydride having the least percentage composition (0.62) while there were 22 phytochemical compounds. Eleven of those phytochemical compounds are bioactive including hexadecanoic acid, 3-deoxy- d-mannonic lactone, 1,2-benzenedicarboxylic acid, butoxyacetic acid, cis-vaccenic acid, propionic acid, 6-octadecanoic acid, 4-thiazolidinone, Oxazole, cis-13-octadecanoic acid, oleic acid.

Table 1: Chemical constituents of *Persea americana* seed extract identified via GC-MS.

PEAK	RT	COMPOUND NAME	CHEMICAL FORMULA	COMPOSITION
1	11.877	(R*,R*)-5-Hydroxy-4-methyl-3-heptanone	C ₇ H ₁₄ O	22.82
2	12.460	Decanoic acid	C ₁₀ H ₂₀ O ₂	5.40
3	12.699	Butoxyacetic acid- carboxylic	C ₆ H ₁₂ O ₃	0.99
4	14.435	Ether, 6-methylheptyl vinyl	C ₁₀ H ₂₀ O	6.34
5	14.527	Docosyl octyl ether	C ₃₀ H ₆₂ O	1.73
6	14.703	3-Deoxy-d-mannonic lactone	C ₆ H ₁₂ O ₆	2.49
7	15.474	Oxazole	C ₃ H ₃ NO	2.44

8	16.982	Hexadecanoic acid	$C_{16}H_{32}O_2$	2.90
9	17.486	1,2-Benzenedicarboxylic acid	$C_8H_6O_4$	1.17
10	17.621	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	12.10
11	18.208	1-Octenylsuccinic anhydride	$C_{12}H_{18}O_3$	0.62
12	18.800	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	5.40
13	19.102	1H-Cycloprop[e]azulene decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. alpha., 4beta., 4a. beta., 7. beta., b7a. beta., 7b.alpha.)]-	$C_{15}H_{26}$	6.18
14	19.425	cis-Vaccenic acid	$C_{18}H_{32}O_2$	12.62
15	19.604	Oleic Acid	$C_{18}H_{34}O_2$	5.64
16	19.915	Oleic Acid	$C_{18}H_{34}O_2$	1.63
17	20.268	7-Pentadecyne	$C_{15}H_{28}$	2.33
18	20.564	2-Propenoic acid	$C_3H_4O_2$	0.70
19	23.602	6-Octadecenoic acid	$C_{18}H_{34}O_2$	2.60
20	23.931	4-Thiazolidinone	C_3H_5NOS	1.54
21	23.110	Glyceric acid	$C_3H_6O_4$	1.24
22	29.714	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	$C_{16}H_{50}O_7Si_8$	0.95

RT= Retention Time

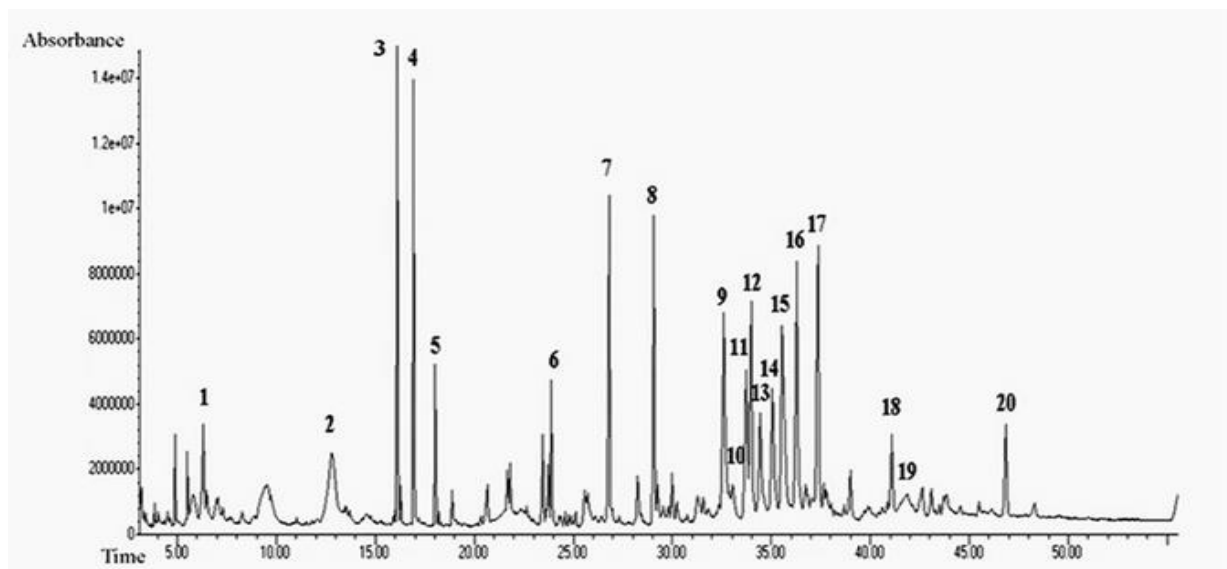


Figure 1: Chromatograph showing the GC-MS analysis of methanol extracts of avocado seed.

Table 2: Acute (oral) toxicity study of Wistar rats after 24h administration of *Persea Americana* Methanol seed extract.

Group	Dose (mg/kg)	Death/number of animals
Control	0	0/5
<i>Persea Americana</i> extract	500	0/5
	1000	0/5
	2000	0/5
	3000	0/5
	4000	0/5
	5000	0/5

Table 2 shows the result of acute toxicity of ESEPA on experimental animals following a 24hr administration. No Behavioural changes or mortality was seen in the rats

when administered with varying doses of ESEPA ranging from 500mg/kg body weight to 5000mg/kg body weight.

Table 3: Changes in Body Weight of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Treatment groups	Pre-induction body weight (g)	7 days post-induction body weight (g)	14 days post-treatment body weight (g)	Change in Weight (g)	Percentage weight change
Normal control	147.67±20.62 ^a	186.51±16.57 ^{a,b}	198.87±17.80 ^a	↑51.21±13.45 ^a	35.65±11.95 ^a
Diabetic control	178.52±22.73 ^b	184.40±23.81 ^{a,b}	174.44±31.47 ^a	↓4.08±0.37 ^b	1.04±0.55 ^b
Diabetic + Glucophage (50 mg/kg)	187.52±19.73 ^b	196.24±14.87 ^b	191.12±22.89 ^a	↑3.61±0.32 ^b	3.03±0.78 ^b
Diabetic + Extract 200 mg/kg	184.45±8.69 ^b	200.55±13.50 ^b	198.69±13.87 ^a	↑14.24±1.87 ^b	7.74±0.61 ^b
Diabetic + Extract 400 mg/kg	181.32±10.92 ^b	174.27±25.46 ^{a,b}	177.46±27.21 ^a	↓3.87±0.35 ^b	1.77±0.65 ^b
Diabetic + Extract 600 mg/kg	176.45±13.19 ^b	159.49±21.02 ^a	178.58±23.46 ^a	↑2.13±0.94 ^b	1.24±0.15 ^b

Values are mean± standard deviation, n=6: mean across the columns with different alphabetical superscripts indicate a significant difference (p<0.05).

Changes in body weight following administration of ESEPA in rats is summarized in table 3. As seen in group 3 animals, animals that received 200mg/kg and

600mg/kg of ESEPA showed a weight increase while the weight of the negative control animals decreased.

Table 4: Blood Glucose Levels of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Group ID	Treatment groups	Post-induction glucose level (mg/dl)	7 days post-induction glucose level (mg/dl)	14 days post-treatment glucose level (mg/dl)	Fall in blood glucose level (mg/dl)	Percentage fall in blood glucose level
I	Normal control	118.00±6.94 ^a	105.20±5.76 ^a	100.20±7.92 ^a	17.80±7.56 ^{a,b}	15.01±6.24 ^{a,b}
II	Diabetic control	313.00±8.66 ^b	316.00±12.58 ^{a,b}	309.40±8.55 ^c	3.60±1.77 ^a	2.46±0.56 ^a
III	Diabetic + Glucophage (50 mg/kg)	290.00±9.58 ^b	316.60±5.32 ^b	191.80±8.66 ^{a,b}	98.20±9.63 ^{b,c}	34.87±9.51 ^{b,c}
IV	Diabetic + Extract 200 mg/kg	298.40±8.83 ^b	221.40±9.60 ^{a,b}	118.40±5.60 ^a	180.00±7.63 ^c	59.15±6.94 ^c
V	Diabetic + Extract 400 mg/kg	321.60±5.35 ^b	300.80±8.39 ^b	231.00±7.51 ^b	90.60±6.81 ^{a,b,c}	28.18±4.41 ^b
VI	Diabetic + Extract 600 mg/kg	320.80±3.69 ^b	242.80±7.12 ^{a,b}	184.60±4.60 ^{a,b}	136.20±5.58 ^c	42.16±4.29 ^{b,c}

Values are presented as mean ±SD, n= 6. Values with different superscript across the columns are significantly different at p<0.05.

Table 4 showed the effects of ESEPA on blood glucose levels of alloxan induced diabetic rats. Animals that received maximum dosage of ESEPA showed a

percentage reduction in blood glucose level statistically comparable (p<0.05) to the rats that received standard drug, Glucophage.

Table 5: Haematological Parameters of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Treatments	Group I	Group II	Group III	Group IV	Group V	Group VI
RBC x10 ⁶ /mm ³	6.28±0.23 ^c	5.18±0.08 ^a	5.90±0.28 ^b	5.93±0.12 ^b	6.37±0.10 ^c	6.42±0.10 ^c
PCV (%)	44.00±1.00 ^c	36.40±2.07 ^a	41.80±1.79 ^b	42.00±1.23 ^b	44.40±0.55 ^c	44.40±0.55 ^c
Hb (g/dl)	16.22±0.23 ^c	13.92±0.23 ^a	15.36±0.62 ^b	15.44±0.30 ^b	16.12±0.19 ^c	16.16±0.15 ^c
WBC x10 ³ /mm ³	9.25±0.21 ^a	12.72±0.66 ^c	11.29±1.06 ^b	10.98±0.58 ^b	10.07±0.45 ^a	9.95±0.44 ^a
PLT x10 ³ /mm ³	230.20±3.77 ^a	257.40±11.72 ^c	244.00±5.61 ^b	241.20±7.16 ^b	237.80±2.59 ^{a,b}	235.20±3.42 ^{a,b}
MCV (pg)	70.08±1.61 ^a	70.19±3.11 ^a	70.86±0.79 ^a	70.86±0.76 ^a	69.75±0.85 ^a	69.19±0.92 ^a
MCH (fl)	25.40±0.64 ^{b,c}	26.85±0.29 ^d	26.04±0.46 ^c	26.06±0.32 ^c	25.33±0.46 ^{a,b}	25.18±0.32 ^a
MCHC (g/dl)	36.87±0.34 ^a	38.32±1.67 ^b	36.76±0.72 ^a	36.77±0.63 ^a	36.31±0.24 ^a	36.40±0.25 ^a

Values are presented as mean ±SD, n= 6. Values with different superscript across the rows are significantly different at p<0.05. RBC, Red Blood Cells; PCV, Packed Cell Volume; PLT, Platelet; Hb, Haemoglobin; MCV, Mean Corpuscular

Volume; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; WBC, White Blood Cell.

Test animals in table 5 showed a statistically significant increase ($p < 0.05$) in the levels of RBC, PCV and Hb when compared with the group 2 animals that served as negative control. However, the levels of WBC and

platelets significantly reduced when the group 2 animals are compared with both the normal control group and extract tested groups.

Table 6: Renal Functions Parameters of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Treatment groups	Group I	Group II	Group III	Group IV	Group V	Group VI
Urea (mg/dl)	19.52±0.53 ^a	28.42±1.22 ^c	21.42±0.68 ^b	21.15±0.99 ^b	21.72±1.21 ^b	21.99±1.92 ^b
Creatinine (mg/dl)	0.84±0.05 ^a	1.20±0.07 ^c	0.96±0.06 ^b	0.87±0.05 ^a	0.91±0.03 ^{a,b}	0.85±0.04 ^a
Na ⁺ (mEq/L)	130.72±1.01 ^c	124.34±0.69 ^a	126.86±0.72 ^b	127.64±1.51 ^b	129.80±1.74 ^c	129.75±1.85 ^c
K ⁺ (mEq/L)	4.57±0.04 ^c	4.24±0.07 ^a	4.26±0.05 ^a	4.35±0.03 ^b	4.38±0.08 ^b	4.36±0.07 ^b
Cl ⁻ (mEq/L)	90.17±0.88 ^d	81.94±1.66 ^a	84.18±0.94 ^b	86.21±1.33 ^c	89.26±1.95 ^c	89.29±2.02 ^c
HCO ₃ ⁻ (mmol/L)	19.80±0.35 ^a	20.80±0.07 ^b	20.10±0.20 ^a	19.92±0.28 ^a	19.85±0.28 ^a	19.87±0.33 ^a

Values are presented as mean ±SD, n= 6. Values with different superscript across the rows are significantly different at $p < 0.05$.

Results on table 6 show the effect of ESEPA on renal function tests in diabetic rats. When compared to the negative control group, levels of urea and creatinine significant decreased ($p < 0.05$). However, no significant

alterations ($p > 0.05$) was recorded in the level of HCO₃⁻ when treated rats were compared to normal control group.

Table 7: Liver Functions Parameters of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Treatment groups	Group I	Group II	Group III	Group IV	Group V	Group VI
Total protein (g/dl)	5.38±0.22 ^b	4.29±0.09 ^a	5.19±0.09 ^b	5.41±0.07 ^b	6.03±0.15 ^c	5.89±0.25 ^c
ALT (u/l)	41.20±3.11 ^a	76.80±3.70 ^d	48.20±1.79 ^b	48.40±4.72 ^b	54.00±4.18 ^c	53.20±2.95 ^c
AST (u/l)	52.20±2.59 ^a	94.80±3.56 ^c	64.40±3.05 ^c	69.80±2.95 ^d	72.60±5.03 ^d	59.20±5.40 ^b
ALP (u/l)	67.00±2.55 ^a	88.60±5.60 ^b	69.40±3.21 ^a	66.60±2.30 ^a	66.40±2.70 ^a	65.60±3.51 ^a
Albumin (g/dl)	3.19±0.07 ^c	1.97±0.07 ^a	2.87±0.20 ^b	3.03±0.13 ^c	3.18±0.09 ^c	3.16±0.06 ^c
Globulin (g/dl)	2.19±0.19 ^a	2.31±0.14 ^a	2.32±0.20 ^a	2.38±0.09 ^a	2.85±0.14 ^b	2.74±0.21 ^b
Bilirubin (mg/dl)	0.50±0.03 ^a	0.84±0.08 ^c	0.62±0.05 ^b	0.58±0.03 ^b	0.60±0.07 ^b	0.61±0.04 ^b
Conjugated bil. (mg/dl)	0.15±0.14 ^a	0.52±0.21 ^c	0.24±1.43 ^b	0.21±2.01 ^b	0.26±0.71 ^b	0.28±0.17 ^b

Values are presented as mean ±SD, n= 6. Values with different superscript across the rows are significantly different at $p < 0.05$. AST, aspartate transaminase; ALT, alanine transaminase; ALP, alkaline phosphatase.

Effects of ESEPA on liver function parameters on diabetic rats are shown on table 7. Results show a significant elevation ($p < 0.05$) in the levels of total protein, albumin and globulin when ESEPA treated groups are compared to the group II animals (negative control animals). These changes are more predominant in animals that received 400mg/kg and 600mg/kg of

ESEPA. However, animal treatment with the various doses of ESEPA led to a significant decrease ($p < 0.05$) in the levels of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total and conjugated bilirubin in the test groups when compared to the negative control group.

Table 8: Lipid profile parameters of Rats Treated with Ethanol Seed Extract of *P. americana* on Alloxan Induced Diabetic rats.

Treatment groups	T. Chol. (mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Group I	95.29±2.99 ^a	59.05±1.35 ^b	76.83±3.19 ^a	20.87±3.10 ^a	15.37±0.64 ^a
Group II	116.48±3.13 ^d	54.05±0.85 ^a	94.21±4.03 ^b	43.59±3.46 ^d	18.84±0.81 ^b
Group III	103.53±3.71 ^{b,c}	55.76±1.85 ^a	79.94±2.19 ^a	31.78±3.73 ^c	15.99±0.44 ^a
Group IV	106.59±4.58 ^c	60.36±2.76 ^b	79.65±1.49 ^a	30.30±5.42 ^{b,c}	15.93±0.30 ^a
Group V	105.73±2.04 ^c	63.87±1.55 ^c	78.55±3.20 ^a	26.14±2.95 ^{a,b}	15.71±0.64 ^a
Group VI	100.53±4.21 ^b	63.22±1.59 ^c	79.75±3.30 ^a	21.36±4.82 ^a	15.95±0.66 ^a

Values are presented as mean ±SD, n= 6. Values with different superscript across the columns are significantly different at $p < 0.05$. Tchol, Total Cholesterol; HDL-C, High-Density Lipoprotein Cholesterol; Triacylglycerol, LDL-C, Low-Density Lipoprotein Cholesterol; VLDL-C, Very Low-Density Lipoprotein Cholesterol.

Table 8 is the effects of ESEPA on lipid profile of alloxan induced diabetic rats. Levels of total cholesterol (T. chol), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) decreased significantly when the

treated rats were compared to negative control groups. A significant rise in the level of high-density lipoprotein cholesterol (HDL-C) was seen when ESEPA treated rats were compared to the normal control group.

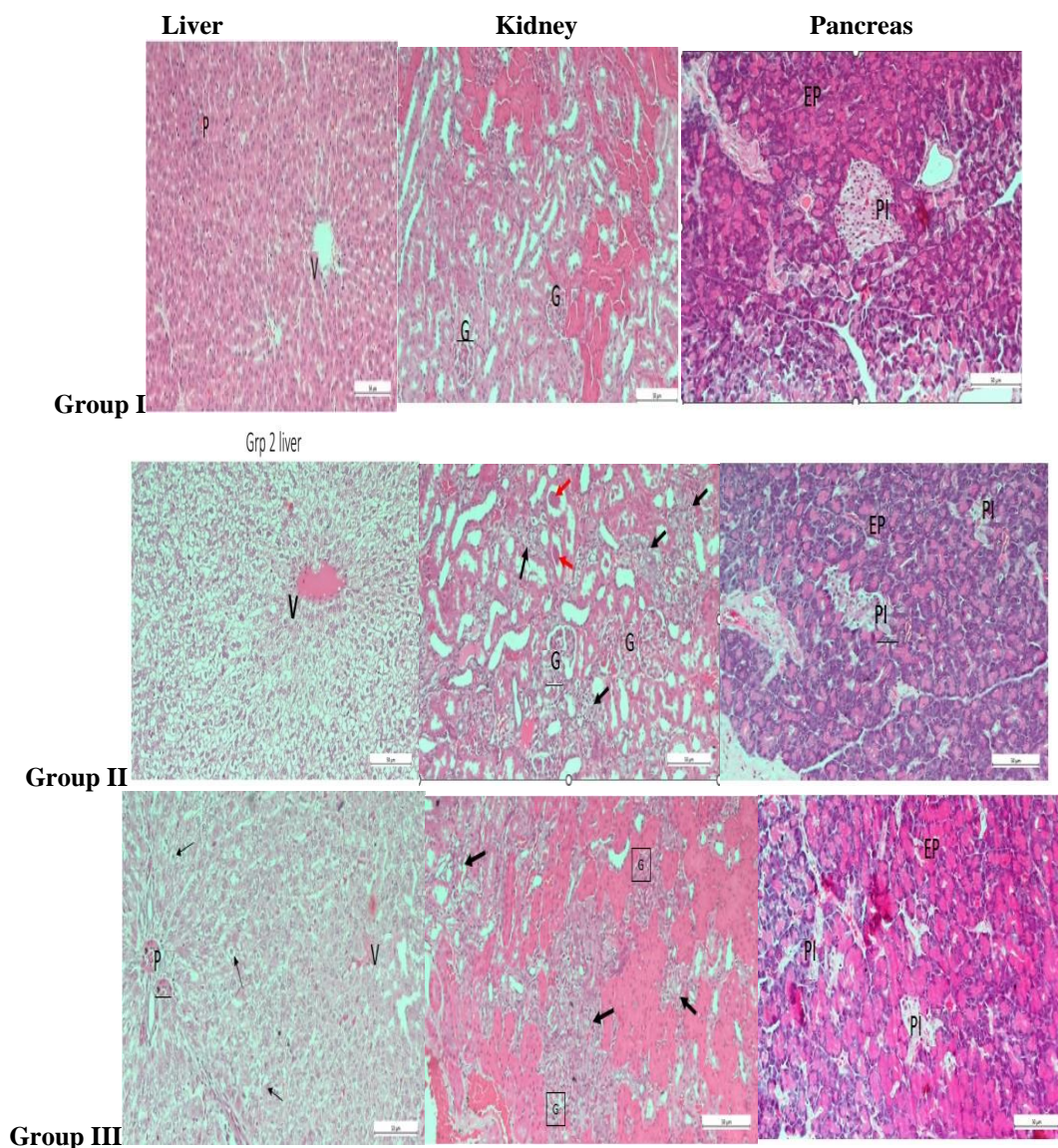
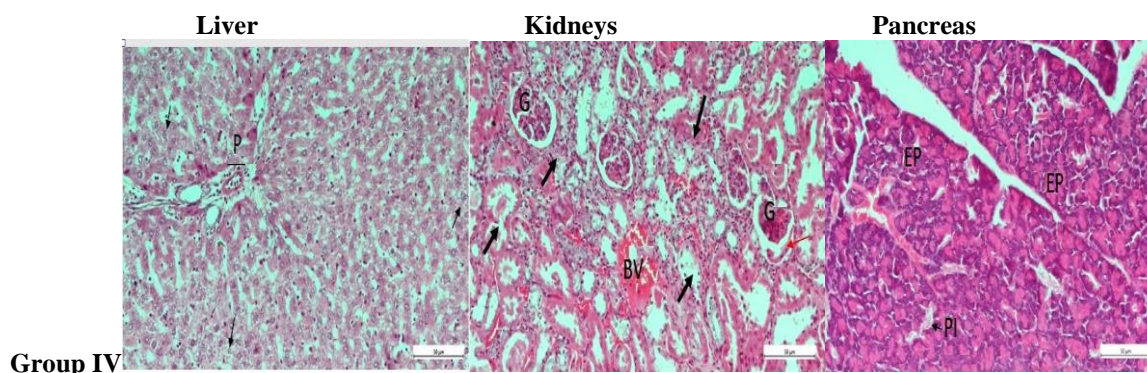


Figure 2: Histological Effects of Ethanol Seed Extract of *P. americana* on the liver, kidneys and Pancreas.

Haematoxylin and eosin staining (H), magnification x 100

In the liver, V= central vein; P = portal triad. In the kidney, G= glomerulus. In the pancreas, PI= pancreatic islets, EP= endocrine pancreas.



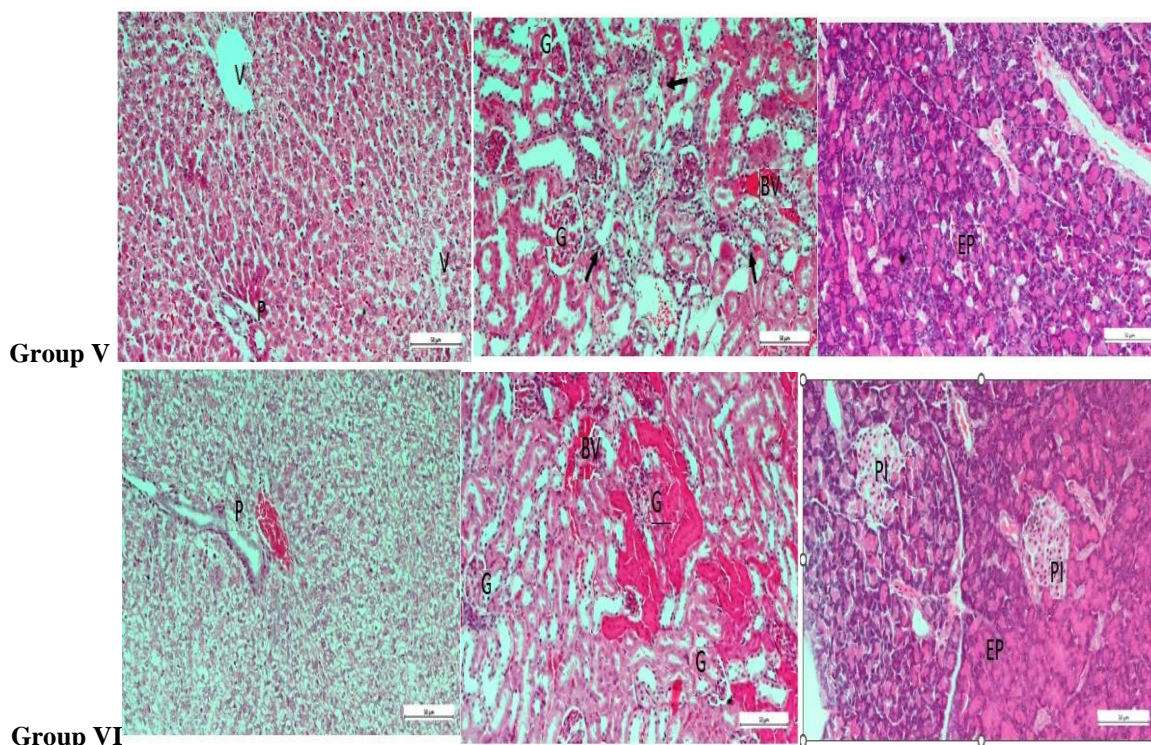


Figure 3: Histological Effects of Ethanol Seed Extract of *P. americana* on the liver, kidneys and Pancreas.

Haematoxylin and eosin staining (H), magnification x 100.

In the liver, V= central vein; P = portal triad. In the kidney, G= glomerulus, BV= blood vessel. In the pancreas, PI= pancreatic islets, EP= endocrine pancreas.

When the histopathological micrographs of the treated rats were compared with both negative and positive control groups, it was obvious that the ESEPA extract did not induce any deleterious effects on the kidneys, liver and pancreas of the tested animals. This attests to the non-toxicity of the ESEPA on the architecture of the liver, pancreas and kidneys.

DISCUSSION

Recent years have witnessed a shift towards the use of phytomedicine as against orthodox medications in the management of various diseases especially those without officially established curative treatment such as diabetes mellitus.^[15] The leaves and fruits of *Persea Americana* have previously been shown by various researchers to contain phytochemicals effective against hyperglycaemia, obesity, pain, arthritis and a form of blood cancer called acute myelogenous leukemia.^[22,23] Different parts of avocado pear and other plants are therefore employed as raw materials for the formulation of different classes of drugs, be it anticancer, antimalaria, antifungal, antiviral and antiulcer medications.^[24,25] This preference for the prevalent use of herbal formulations is hinged on the fact that the bioactive compounds present in these plants cause little or no side effects and complications, these plants are relatively available, accessible and affordable to the folks that use them. The

present study therefore aimed to assess the antidiabetic properties of *P. Americana* seed and to determine the safety margin of this extract in organs such as the liver, kidneys and pancreas after phytochemical characterization.

The result of the Gas Chromatography Mass Spectrometry (GCMS) analysis identified twenty-two phytocompounds present in the ESEPA, out of which eleven have established potent pharmacological capabilities. For instance, hexadecanoic acid is antimicrobial, anti-inflammatory, anticovid-19 and cytotoxic,^[26,27,28] 3-deoxy- d-mannonic lactone is antimicrobial, anti-inflammatory, cytotoxic, antiviral and antiparasitic,^[29,30] 1,2-benzenedicarboxylic acid has cytotoxic and antimicrobial properties,^[31,32] butoxyacetic acid is a haemolytic agent,^[33] cis-vaccenic acid has anti-obesity, anti-tumor, antidiabetic and enhanced insulin sensitivity.^[34,35,36,37] Also propionic acid is antibacterial and antifungal,^[38] 6-octadecanoic acid has antidiabetic, wound healing, anticovid-19, antistomatitis and antiperitonitis effects,^[39] 4-thiazolidinone possess anti-inflammatory, antiproliferative, analgesic, antiviral, anticonvulsant, antidiabetic, antihyperlipidaemic, cardioprotective, antitubercular, antifungal and antibacterial activities,^[40,41] while Oxazole is anticancer, antiviral, anti-inflammatory, antibacterial, antifungal, antidiabetic, antitubercular, antiparasitic, anti-neuropathic, anti-obesity, antioxidative and analgesic.^[42,43] Entigu et al,^[44] reported antiviral activities of cis-13-octadecanoic acid while oleic acid has been shown to be hepatoprotective, antihypertensive and anticancer.^[45,46]

Acute toxicity study involves the treatment of animals with varying doses of a plant extract to animal models to establish the safety of such a plant material before human trial.^[12,47] In this study, experimental rats did not show any features of acute toxicity and no deaths were recorded when the animals were exposed to the graded doses of ESEPA (500mg/kg to 5000mg/kg). From our findings, the LD₅₀ of the ESEPA is greater than 5000mg/kg and is safe, non-toxic and can be used for therapeutic purposes.^[48,49] Besides mortality and behavioral changes, changes in body weight of animals serve as viable indicator of exposure of animals to toxic substances. Previous findings have shown that body weight loss may be used to calibrate the level of toxicity of a plant material.^[50] In the index study, animals treated with ESEPA showed a steady healthy increase in weight throughout the course of the experiment implying that ESEPA is non-toxic.

The hypoglycaemic and antihyperglycaemic effects of ESEPA in alloxan induced diabetic animals were confirmed by progressive reduction in blood glucose level of animals that received ESEPA. The 14-day percentage fall in blood glucose level seen in rats that received 400mg/kg of extract is comparable to the levels seen in animals that received the standard oral antidiabetic drug, glucophage. Our findings in the study is in tandem with those of Zofou et al.^[15] and Ezejiofor et al,^[51] where ingestion of varying doses of *P. americana* extract led to a decrease in blood sugar of rats. Bioactive compounds seen in our GCMS analysis such as vaccenic acid, 6-octadecanoic acid, 4-thiazolidinone, oxazole present in *P.americana* seed extract possess potent antidiabetic activities and increase insulin sensitivity in animal cells as have been previously described by Jahreis et al,^[37] Tripathi et al,^[40] Arundina et al.^[39] Also, the seed extract did not deleteriously affect the pancreas of treated rats. Also, Anita et al,^[52] reported a 60% drop in blood glucose level of experimental rats, 6 hours after treatment with *P. americana* leaf extract.

Hematological indices are viable indicators for studying the adverse effects of drugs and other xenobiotics as well as disease (anemia, thrombocytopenia, leucopenia) monitoring. Blood parameters such as MCV, HB, PCV, RBC, MCH and MCHC are often used to monitor animal and human progress during treatment for anemia.^[12] Treatment with ESEPA did not disrupt the haematopoietic machinery of treated rats as evidenced by increase in the levels of RBC, PCV and Hb in animals that received the extract. Antioxidant capability of ESEPA may explain its blood enhancing effects since free radical attack has been previously linked to the impairment of haematopoietic process.^[53]

Alterations in biochemical indices of the liver and kidneys serve as an important assessment tool in determining extract efficacy and toxicity in experimental animals. Serum urea, creatine and electrolytes such as potassium, sodium, chloride and bicarbonate are all

dependable markers of kidney dysfunction.^[54] Increased serum urea and creatine are diagnostic of kidney pathology.^[9] In the index study, treatment of experimental rats with various doses of ESEPA resulted in a decrease ($p<0.05$) in the level of Urea and Creatine and did not adversely affect the level of the HCO_3^- . The histomorphology of the kidneys did not exhibit any deleterious lesions, implying that the ESEPA is not nephrotoxic.

The liver is the primary organ for drug metabolism/detoxification, metabolism of haem, bile secretion, storage of iron and synthesis of biomolecules. ALT, AST and ALP, bilirubin both total, conjugated and unconjugated increase in levels when the hepatocytes are damaged or become necrotic.^[24,55] Primary liver cell carcinoma, viral and paracetamol induced hepatotoxicity, liver cirrhosis, fatty liver disease and fibrosis of the liver are associated with concomitant elevation of ALT, AST, ALP and bilirubin.^[56] Experimental animals treated with 400mg/kg and 600mg/kg of ESEPA showed an elevation in the level of total protein, albumin and globulin, while ALT, AST, ALP and bilirubin significantly decreased lending credence to the hepatoprotective nature of *Persea americana* as reported by Kawagishi et al,^[57] Husena et al,^[58] Salgado et al,^[59] Stucker et al,^[60] and Tango et al.^[61] Oleic acid reported in our GCMS analysis has previously been described as hepatoprotective.^[45,46] Also the histology of the Liver of the treated rats did not show any pathological lesion.

Dietary modifications and lifestyle changes such as exercise are among the effective ways of managing dyslipidaemia. Stroke, coronary artery disease and high blood pressure are seen to complicate hyperlipidaemia.^[62] Hyperlipidaemia is associated with elevated levels of serum cholesterol, triacylglycerol, phospholipids and alterations of lipoprotein levels.^[50] While elevated HDL protects against coronary heart diseases, LDL, VLDL and serum cholesterol when elevated predisposes to cardiovascular diseases. The data obtained in this study clearly indicated that administration of ESEPA triggered antihyperlipidaemic effect in experimental rats by decreasing the levels of total cholesterol, triacylglycerol, low density lipoprotein cholesterol and very low density lipoprotein while the level of HDL increased. 4-thiazolidinone reported in the GCMS analysis in this experiment has been previously identified by Tripathi et al,^[40] and Roszczenko et al,^[41] to be hypolipidaemic. Our findings are also similar to those of Pliego and Litz,^[63] and Brai et al,^[64] who reported the antihyperlipidaemic effect of *P. americana* leaf extract.

CONCLUSION

The index study showed that the analysis of ESEPA identified oxazole, vaccenic acid, 6-octadecanoic acid and 4-thiazolidinone which exhibit potent pharmacological activities such as antidiabetic, enhanced insulin sensitivity, hypolipidaemic, hepatoprotective and antioxidant effects. From our findings, oral

administration of ESEPA did not produce any adverse complications such as weight loss, organ damage, behavioral changes or other signs of toxicity. Also no mortality was registered in both acute and subacute toxicity studies. The study also demonstrated that ESEPA is hepatoprotective, renoprotective and has antidiabetic capabilities and do not adversely affect haematological and histological indices. From, the foregoing, there is therefore a scientific basis for the use of ESEPA in the treatment of hyperglycaemia. We recommend a chronic study on the safety margin of ESEPA and a human clinical trial. Further research is also needed to elucidate the antidiabetic mechanism of action of ethanol seed extract of *Persea americana*.

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