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Effect of Aqueous Extract of *Persea americana* Seed on Blood Glucose in Alloxan-induced Diabetic Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAA and RNA designed the study. Author MEA performed the statistical analysis and literature searches. Author OMO wrote and monitored the first draft of the manuscript. Author JCI supervised the entire process and author ME performed the bench work. All authors read and approved the final manuscript.

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ABSTRACT

Alternatively known as grape sugar, Glucose, a monosaccharide ($C_6H_{12}O_6$) is often found in honey and the juices of many fruits. It is the sugar most often produced by hydrolysis of complex compounds from plants (natural glycosides) and forms a major constituent of the blood of animals. One of the major implications of increased blood glucose is Diabetes Mellitus. In spite of its life-threatening complications, the issue of cost, and appropriate dosage administration (with synthetic

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drugs) remain an issue. This study was undertaken to determine the hypoglycemic (glucose lowering) activity of *Persea americana* seed extract in the blood of alloxan-induced, diabetic (hyperglycemic) wistar rats. To achieve this, Thirty-Five (35) Wistar rats (140 – 200 g) were assigned into seven groups of 5 rats each with group A serving as normal control. While Groups B through E were respectively treated with 250 mg/kg and 500mg/kg of Persea seed extract (Non-diabetic rats), Alloxan (diabetic control), and Alloxan (diabetic, then treated with 50 mg/kg of metformin), Groups F and G were diabetic rats which respectively received 250 mg/kg and 500mg/kg of Persea seed extract. After 28 days of administration, rats were sacrificed with blood samples collected for analysis of glucose levels. Pancreatic tissues were also harvested for histopathological extermination. Results show an increase in body weight (20.39 – 26.32%) of diabetic treated rats (group E) as compared to diabetic control (Group D). Though blood glucose levels for group E reduced significantly (at $p<0.05$) in a dose-dependent manner as compared to elevated levels in diabetic control, no significant changes were observed in normal treated rats (group D) as against normal control (Group A). Histopathology of the pancreas revealed a degeneration in the islet cells of diabetic untreated groups F and G compared to non-diabetic group A.

Keywords: Alloxan; diabetes; hyperglycaemia; hypoglycaemia; blood glucose.

1. INTRODUCTION

Diabetes Mellitus (DM) is old with man. It is one of the world's leading causes of death with over 150 million new cases reported yearly [1]. Some available findings posit that over 1.70 million of such global cases are seen in Nigerians above 15 years old; with about 70,000 of them occurring as type I in children under the ages of 15 years [2]. Diabetes is prevalently rising daily as a result of obesity, population growth, and sedentary lifestyles; projecting it to be over 360 million cases by 2030 [3]. Though efforts have been made to prevent its life threatening complications through the use of oral hypoglycemic (blood sugar reducing) agents like sulphonylureas, Diabetes remains an incurable endocrine disorder. Cost, undesirable and adverse effects associated with these drugs promote the use of suitable herbs with minimal effect hypoglycemic activities. Over 50% of such herbs now serve traditional medics in combating and ameliorating ailments like dysentery, diarrhea, toothache, skin infections, and diabetes; which greatly affect humans. One of such plants often alleged to be of great importance is *Persea americana* (Avocado).

Native to both Mexicans and Americans*, Persea americana* is a tree of the Lauraceae family. It is widely cultivated in the subtropical and tropical countries of the world with an average height of about 8-9m long, posing as evergreen leaves [4,5]. Dicotyledon of Avocado seeds are used in Mexican and African ethno-medicine as potent remedy against ailments as muscle pains, menstrual disturbances as well as diabetes [6,7].

In recent times, Avocado has been reported to be non-toxic to humans. However, its leaves, fruits and skin are poisonous to some animals; cats, dogs, cattles, goats, birds, fishes, and horses [8]. Avocado seeds, leaves and fruits contain such phytochemicals as; alkaloids, tannins, flavonoids, triperpenes, carotenoids, phenols, cellulose, sateroids, polyuronoids, b-galactoside and volatile oil [9,10,11]. Avocado contains bursts of nutrients like Vitamins (B, E, and K), potassium, calcium, magnesium, iron, dietary fibres and mono-unsaturated fatty acids [12]. Its seed serves as anti-oxidants in some traditional foods due to its anti-oxidative properties [13]. The efficacy of its leaf in the management of infants' convulsion has been reported [14]. Its constituent carotenoids and tocopherol have been reported to inhibit growth of cancerous cells in the prostate gland [5]. Anti-inflammatory, analgesic, and hepatoprotective effects of the plant have also been reported in animal models [7,10]. The existing management of Diabetes Mellitus using orthodox and various medications is expensive, requiring prolong use and proper storage facilities with serious side effects (Renal, Hepatic and Hematological) as accomplice. Herbal remedy seems assessable and inexpensive with little or no side effects.

This study aimed at ascertaining the hypoglycaemic activity of *Persea americana* seed aqueous extract on alloxan induced diabetics, using Wistar rats as experimental model. Specifically, Study attempted to:

i. Access the effect(s) that Avocado seed extract has on the body weights of hyperglycaemic rats.

ii. Access the blood sugar lowering activity of the Avocado seed extract on hyperglycaemic rats
iii. Examine the extract's activity on the pancreas histology.

2. MATERIALS AND METHODS

2.1 Scope of Study

Study was conducted in the Department of Human Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.

2.2 Materials

2.2.1 Plant sample collection and identification

Fresh fruits of *Persea americana* were obtained from Osa street in Agbor, Delta State. They were then authenticated by taxonomists in the Department of Botany, Faculty of Sciences, Delta State University, Abraka, Delta State, Nigeria.

2.2.2 Preparation of plant's extract

The fruits were cut opened with a sharp knife to harvest its seeds. These seeds were then chopped in small pieces and sun-dried for two days. They were then mashed into coarse powder, using mortar and pestle. 500g of the powder was then soaked in 1.5 liters of distilled water for 48 hours and then filtered with cheesecloth sieve. Filtrate (aqueous extract) was then concentrated in a rotary evaporator and further dried in an oven of 3^0C for 3 days. Yielded extract was then stored within an air-tight bottle in a refrigerator (4^0C) until required for the study.

2.2.3 Chemical reagents

The chemicals (analytical grade) for this study were procured from Sonitex Enterprise, Benin City, Edo State. These reagents were commercial kits and products of TECO Diagnostics, Anaheim, and included Metformin (Glycophage). The tablets were dissolved in distilled water to obtain the appropriate concentration for administration. 10% of formaldehyde and Alloxan-monohydrate (Batch number 26027) solutions were obtained from Department of Histopathology section of Emma-Maria Biomedical Laboratory, Abraka.

2.2.4 Ethical clearance

Ethical clearance was obtained from the Research and Ethics Committee of the Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State. All animals were treated in line with guidelines, stipulated by the National Institute for Health Guide on the Care and Use of Laboratory Animals (1985).

2.2.5 Experimental animals

Thirty five (35) healthy Wistar rats (between 140-200 g) were procured from Emma-Maria Laboratory, Animal House unit, Abraka, Delta State. The animals were transported in plastic baskets to the animal house of the College of Health Sciences, Delta State University, Abraka, Delta State. They were then housed in wire mesh cages and given 2 weeks to acclimatize under standard laboratory conditions.

2.2.6 Acute toxicity test

Available acute toxicity study suggests that Oral medium lethal dose (LD_{50}) of aqueous seed extract of *Persea americana* is greater than 10,000 mg/kg body weight[8]. More so, according to the American Society for Testing and Materials (1987), any chemical substance with LD_{50} estimate greater than 3,000-5,000 mg/kg (Oral route) could be considered of low toxicity and safe. Based on these, two concentrations (250 mg/kg and 500 mg/kg) of aqueous extract from *Persea americana* were used for the experiment.

2.3 Procedure

Animals were weighed, using digital balance before diabetes was induced. Weighing occurred daily during extract administration and on the day of sacrifice.

2.3.1 Diabetes induction

After 10-12 hours overnight fast, twenty (20) animals were induced with diabetes. This happened through a single intravenous injection of 35mg/kg body weight of alloxan monohydrate, dissolved in normal saline [15]. After one hour of alloxan injection, the rats were given normal feed and water *ad libitum*. The blood glucose concentrations of induced rats were confirmed after 72 hours, using blood glucose meter (Glucometer). Sugar levels ≥ 200 mg/dl were considered diabetic [16].

2.4 Study Design

The animals were assigned into seven (7) groups of five rats each (n=5) with the extract orally administered for 28 days. Here is a breakdown of treatments for each group:

Group A (Normal Control): Given standard rat feed and water.

Group B (Non-Diabetic): Given 250 mg/kg body weight of extract daily, with normal feed and water.

Group C (Non-Diabetic): Given 500 mg/kg body weight of extract daily, with normal feed and water.

Group D (Diabetic Control): fed with normal feed and water after diabetes induction.

Group E (Diabetic): Received 50 mg/kg body weight of Metformin daily, with normal feed and water after diabetes induction.

Group F (Diabetic): Received 250 mg/kg body weight of extract daily, with normal feed and water after diabetes induction.

Group G (Diabetic): Received 500 mg/kg body weight of extract daily, with normal feed and water after diabetes induction.

2.4.1 Sample collection

After an overnight fast, rats were anaesthetized under chloroform vapour. Blood samples were obtained by cardiac puncture using 5 ml syringe and 23G needle. Obtained blood samples were then centrifuged (at 3500 rpm) for 10 minutes to obtain serum for biochemical analysis. Pancreatic tissues were also harvested and stored in a container of 10% formol-saline for fixation and histological photomicrography.

2.5 Statistical Analysis

Statistical significance of treatment effect(s) was analyzed with the students' t-test, with values expressed as Mean ± SEM (Standard Error of Mean). All of these processes were automated and achieved with the statistical package for social sciences (SPSS) version 20. Differences between means were considered at $p<0.05$.

3. RESULTS

See figures and plates below for presentation of tables and micrographs of obtained records.

4. DISCUSSION

The use of plants for treatment of diseases, particularly diabetes mellitus is as old as man. This is because of the potent bioactive compounds with anti-diabetic properties present in these plants [17].

Alloxan monohydrate is one of the numerous chemical agents used to induce diabetes mellitus in animal models. It acts by partial destruction of the Beta cells of the Islet of Langerhans of the pancreas, resulting to decreased insulin secretion and hyperglycaemia [18].

Results from this study revealed a significant loss of body weight of untreated diabetic compared to non-diabetic animals. This may be due to the loss in muscle and adipose tissue, resulting from excessive breakdown of tissue protein and fatty acids [19]. Glycosuria is known to cause significant loss of calories via glucose excretion. Presumably, this loss results in severe weight lost in spite of increased appetite. Severe weight loss was probably prevented in the extract treated group due to interaction of several bioactive constituents. However, the extract treated animals showed appreciable increase in weight compared to the diabetic control group, indicating that the treatment allowed tissues to access the glucose for energy supply, and to build tissue materials required for growth. This is in line with that of Mohammed *et al.,* 2006[17]. Weight gain in non-diabetic rats may be due to proper utilization of food and fluid influenced by extracts.

Blood glucose concentration of animals were significantly seen to have elevate ($p<0.05$) after alloxan injection. Oral administration of aqueous Avocado seed extract produced an insignificant change in blood glucose levels for normal treated rats. The significant decrease in blood glucose of diabetic treated rats may indicate the presence of hypoglycaemic agents in Avocado seed extract. The result suggested that the extract exhibits hypoglycaemic activity, and it is in line with previous reports [20]. The extract could have acted via increased insulin secretion or increased peripheral glucose utilization in the normal treated rats, through stimulation of peripheral glucose utilization in the GIT.

Studies have shown that phytochemicals (flavonoids, saponins, tannins, alkaloids, etc.) in plants act as anti-oxidants and contain insulin stimulating substances such as insulin receptors substrate (IRS), glycogen synthase, glucose

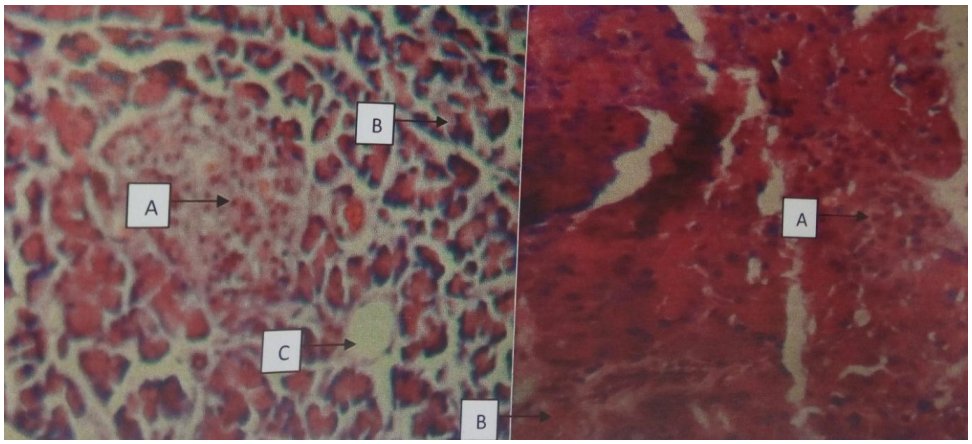

Plate I **Plate II**

Plate I: Normal control rat pancreas composed of endocrine gland (A), surrounded by exocrine glands (B), thrown into lobules by septa (C) (H & E staining x 10).

Plate II: Non-diabetic rat pancreas treated with 250 mg/kg body weight of Persea americana. With mild islet cells (A), surrounded by exocrine glands (B) (H & E staining x10).

Fig. 1. Comparing photomicrograph of pancreas histology for Non-diabetic treated rats (250 mg/kg of extract) to Normal control rats.

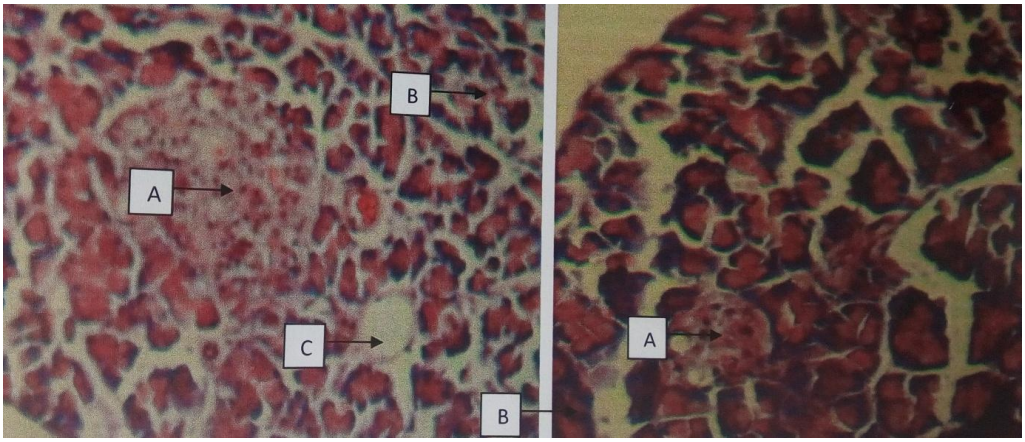

Plate I **Plate III**

Plate I: Normal control rat pancreas composed of endocrine gland (A), surrounded by exocrine glands (B), thrown into lobules by septa (C) (H & E staining x 10).

Plate III: Non-diabetic rat pancreas treated with 500 mg/kg body weight of Persea americana. With islet cells (A), surrounded by exocrine glands (B) thrown into lobules by septa and mild vascular congestion (H & E staining x10).

Fig. 2. Comparing photomicrograph of pancreas histology for Non-diabetic treated rats (500 mg/kg of extract) to Normal control rats

dependent insulinotropic polypeptide (GIP) receptors [21]. These hypoglycaemic agents may have contributed to the observed anti-diabetic activity of the avocado seed extract.

Impaired glucose homeostasis in diabetes increases the volume of extraction of metabolites via the kidney, which results in electrolytes imbalance [19]. Electrolytes play an important

role in many body processes, such as controlling fluid levels, acid-base balance (PH), nerve conduction, muscle contraction, and blood clotting. No significant change(s) was/were observed in weight of the pancreas for diabetic treated and normal rats. This lack of direct organ toxicity may be due to Avocado extract's antioxidant effect of flavonoids that protects organs against such oxidative stress induced damage.

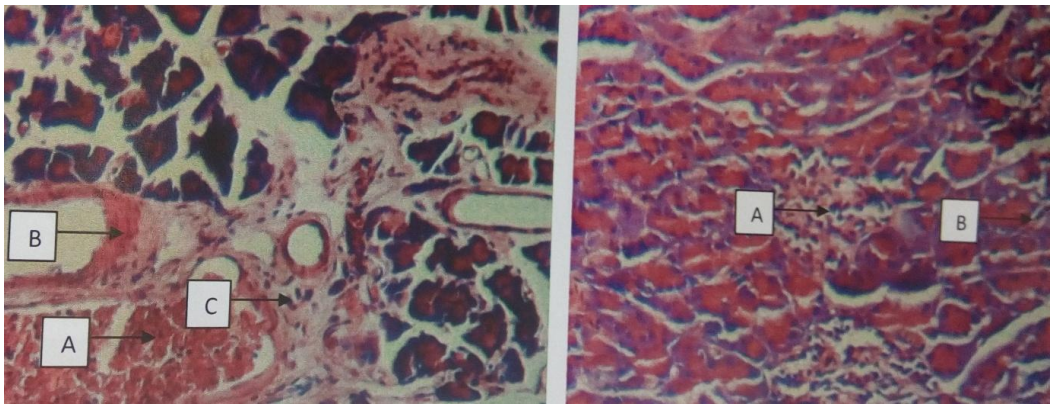

Plate IV **Plate V**

Plate IV: Diabetic untreated rat pancreas with moderate interlobular vascular congestion (A), dilatation and hypertrophy (B) as well as mild interlobular infilterates of chronic inflammatory cells (C) (H & E staining x 10).

Plate V: Diabetic pancreas treated with metformin (50mg/kg body weight) with the presence of fairly remarkable islets (A) and exocrine glands (B) (H & E staining x10).

Fig. 3. Comparing photomicrograph of pancreas histology for metformin treated diabetic rats against diabetic control

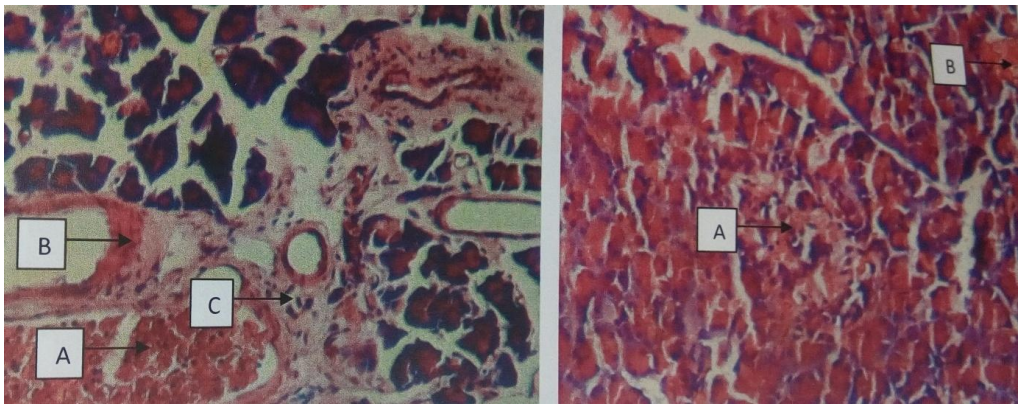

Plate IV **Plate VI**

Plate IV: Diabetic untreated rat pancreas with moderate interlobular vascular congestion (A), dilatation and hypertrophy (B) as well as mild interlobular infiltrates of chronic inflammatory cells (C) (H & E staining x 10).

Plate VI: Diabetic rat pancreas treated with 250 mg/kg of Persea americana with mild resurgent islets (A), and mild vascular congestion (B) (H & E staining x10).

Fig. 4. Comparing photomicrograph of pancreas histology for diabetic treated diabetic rats (250 mg/kg of extract) against diabetic control

Table 1. Effect of *Persea americana* seed aqueous extract on body weight of alloxan-induced diabetic rats

Treatment	Body weight (g)						
	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Initial	146.60±2.86	149.34±1.31	142.50±5.10	171.20±8.45	149.36± 1.97	155.30± 1.38	160.96± 2.10
Final	184.46±5.01	176.16±2.50*	164.70±5.68*	121.25±5.78*	141.02± 2.03	150.86± 3.19	146.82± 4.47*
%Δ.in body weight	25.83	17.96	15.58	-29.18	-5.58	-2.86	-8.79

*Values are expressed as Mean ± SEM, n=5. *P<0.05, final body weight compared to initial body weight.*

Table 2. Effect of *Persea americana* seed aqueous extract on Fasting blood glucose level of diabetic rats

	Fasting blood glucose level (mg/dl)						
	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Initial	88.80±4.32	72.60±3.71	90.40±2.87	528.20±31.35*	495.20± 37.32	465.60± 40.71	461.20± 38.75
Final	104.80±2.52	70.40±5.57	99.40±10.20	254.00±43.82	175.20± 2.25+	255.00± 19.72+	213.20± 18.36+
%Δ.in FBGL	18.02	-3.03	9.96	-15.91	-64.62	-45.23	-53.77

*Values are expressed as Mean ± SEM, n=5. *P<0.05, non-diabetic control compared to other groups, +P<0.05, diabetic treated groups compared to diabetic control group.*

Table 3. Effect of *Persea americana* on relative weight (in kg) of pancreas in alloxan-induced diabetic rats

	Group A	Group B	Group C	Group D	Group E	Group F	Group G
Pancreas	0.64±0.05	0.76±0.05	0.64±0.09	0.58±0.02	0.72± 0.05	0.68± 0.07	0.62± 0.04

Values are expressed as Mean ± SEM, n=5.

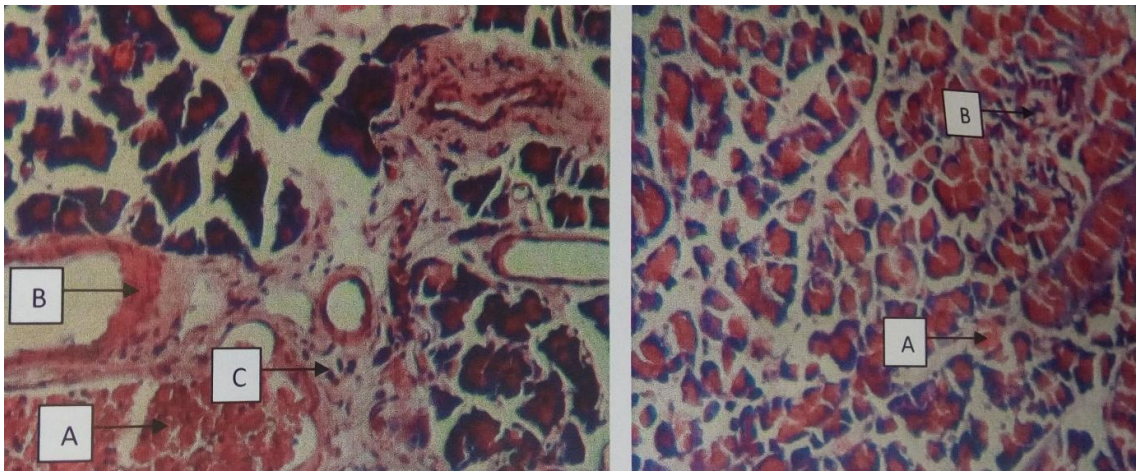

Plate IV **Plate VII**

Plate IV: Diabetic untreated rat pancreas with moderate interlobular vascular congestion (A), dilatation and hypertrophy (B) as well as mild interlobular infiltrates of chronic inflammatory cells (C) (H & E staining x 10).

Plate VII: Diabetic rat pancreas treated with 500mg/kg of Persea americana with faint resurgent islet (A) and mild interlobular vascular congestion (B) (H & E staining x10).

Fig. 5. Comparing photomicrograph of pancreas histology for diabetic treated diabetic rats (500 mg/kg of extract) against diabetic control.

Histopathological study of diabetic untreated rats showed degeneration of pancreatic islet cells, which was due to alloxan monohydrate used in the study. This is in line with previous report[20]. Degeneration of the islet cells probably gave rise to insulin dependency, which caused blood glucose elevation and under-utilization. The histopathological study of diabetic treated groups (D, E and F) induced increased volume density of islet cells and increased beta cells, which may be signs of beta cells regeneration. *P. americana* seed may have some chemical components that exert regenerative effects on beta cells, thus stimulating insulin secretion [7]. Higher dose of the extract has a greater restorative effect on the islet cells of diabetic rats than a lower dose of extract. The tissue-protecting effect of *P. americana* can be observed in its ability to restore and reverse the already damaged tissue of alloxan-induced rats, and the observed effect is in agreement with what was previously reported. There was no significant effect on the pancreas of normal rats.

5. SOCIETAL ADVANTAGE OF STUDY

More than 80% of diabetes deaths occur in low and middle income countries [3]. This is attributed to expensive and inadequate storage facilities for synthetic hypoglycaemic agents. In view of this increased prevalence in diabetes rate, there is need to develop integrated approach towards its management and prevention via exploration of potentials offered by traditional medicine. Synthetic hypoglycaemic agents are expensive, accompanied with serious side effects (haematological effects as well as liver and kidney disturbances). In addition, these synthetic drugs are not suitable for use during pregnancy [22].

6. CONCLUSION

Findings from this study indicate that consumption of aqueous extract from *Persea americana* seed has a preponderant increase in body weight (20.39 – 26.32%) of diabetic treated rats (group E) as compared to diabetic control (Group D). Though blood glucose levels for group E reduced significantly (at $p<0.05$) in a dose-dependent manner as compared to elevated levels in diabetic control, no significant changes was however seen in normal treated rats (group D) as against normal control (Group A)

7. RECOMMENDATIONS

While high level of scepticism exists to explain the actual mechanism of action of the *Persea americana* seed extract;

1. Further work(s) is necessary to isolate the bioactive constituents of Avocado plant's seed for enhanced phytotherapy.
2. Further study with longer period of higher dose may show clearer features of these findings.
3. Possible mechanism(s) of action of Avocado seed extract on electrolytes and glucose homeostasis should be studied.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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