European Journal of Medicinal Plants

26(1): 1-12, 2018; Article no.EJMP.41587
ISSN: 2231-0894, NLM ID: 101583475

Hypoglycaemic and Biochemical Effects of the Aqueous and Methanolic Extract of *Persea americana* Seeds on Alloxan-Induced Albino Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CCE and MA designed the study performed the laboratory and statistical analysis. Author CCE wrote the first draft of the manuscript. Author IEE, supervised the entire work, interpreted the results. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/41587
Editor(s):
(1) Dr. Patrizia Diana, Professor, Department of Molecular and Biomolecular Sciences and Technologies, University of Palermo, Palermo, Italy.
(2) Dr. Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Daniela Hanganu, "Iuliu Hatieganu"University of Medicine and Pharmacy, Romania.
(2) Sudhanshu Kumar Bharti, Patna University, India.
(3) Nyoman Kertia, Gadjah Mada Unversity, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/27303

Original Research Article

Received 06 March 2018
Accepted 17 May 2018
Published 19 November 2018

ABSTRACT

The increase in the prevalence, complications and cost effect of diabetes management necessitated the search for alternative treatment and a look into the anti-diabetic and biochemical effects of *Persea americana* seed extract on the liver of diabetic rats. This study was conceived and designed based on the gaps in the research that has been performed and what is known about the plant. The anti-diabetic and biochemical effects of both the water and ethanol extracts of *Persea americana* (avocado pear) seed on alloxan-induced diabetic albino rats were investigated. The seeds were minced by means of a grater and dried to a constant weight in an oven at 50°C. It is then ground to powder. One hundred grams (100 g) of the sample was extracted with 1000 ml of both water and methanol using the maceration method. The extracts were evaporated to dryness using a rotary evaporator and the extracts stored at 4°C until use. The effects of different doses (200 mg/bw, and 300/bw) of both water and methanol extracts of *P. americana* seed on alloxan-induced diabetic albino rats were compared with those of a reference drug, insulin. The glucose

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level and weight of the rats were measured weekly for 21 days. The liver function tests of the rats were investigated. Results of study showed that the extract possess a significant anti-diabetic effect for both extracts ($P < 0.05$). However, when compared within the experimental groups, the rats treated with ethanol extracts of *P. americana* seed showed a better anti diabetic effect. The liver function enzyme parameters showed no significant difference ($P > 0.05$) and reversed the increased liver function parameters that occurred in alloxan-induced albino diabetic untreated rats, comparable to the effects of insulin. In conclusion, the present study provides a pharmacological basis for the traditional use of *P. americana* seeds extracts in the management of Diabetes Mellitus.

Keywords: P. americana; diabetes; biochemical effects; alloxan; liver function.

1. INTRODUCTION

Diabetes Mellitus is a metabolic disease caused by a deficiency in the secretion or action of insulin [1]. This disorder is characterized by major symptoms as; polyuria (frequent and abundant urine), glycosuria (presence of glucose in urine) and hyperglycemia (glucose rate on an empty stomach higher than 1.2 g/l in plasma blood and confirmed in at least two occasion) [2]. Basically, there are two major clinical classes of diabetes .The type 1 diabetes or insulin dependent diabetes mellitus (IDDM) and type 2 diabetes or non insulin dependent diabetes mellitus (NIDDM) also called insulin resistant diabetes. According to the International Diabetes Federation (IDF) 2014 updates, out of the world seven billion population, 387million people, aged 20–79 years worldwide are diabetic, [3] giving a comparative prevalence of 8.3%, while 46.3% cases are undiagnosed. In every 7 seconds, a person dies of diabetes, 4.9 million deaths was recorded in 2014. Seventy seven percent (77%) of people with diabetes live in low and middle-income countries. Africa has recorded cases of 2,150,274 (5.05%) diabetic patients with over 13 million undiagnosed cases. In Nigeria, there are estimated 374,651 diabetic cases, with another 172,339 undiagnosed cases. These figures account for about 4.64% Nigerian adults between ages 20-79 living with diabetes. An estimated 105,090 Nigerians died in 2014 as a result of diabetes. An average diabetic Nigerian spent about 43527.16 naira (US $178.39) in 2014 due to diabetes treatment [3]. With this alarming prevalence rate, diabetes mellitus poses a major challenge globally and accounts for a number of disabilities and deaths globally.

Medicinal plants have been identified and used throughout human history, [4]. Medicinal Plant materials have been shown to have various chemicals also known as phytochemical at various concentrations. Plants may act on blood glucose through different mechanisms. Some plants may contain insulin-like substances [5], inhibit insulinase activity or increase beta β-cells in the pancreas by activating the regeneration of these cells [6,7], or some may serve as antioxidants by reducing the oxidative stress due to free radicals in the pancreas [8,9].

P. americana is one of the 150 varieties of avocado pear. The pulp of mature avocado fruit has a sweet pleasant taste that is consumed as human food. This fruit is normally used for human consumption, but it also has been used as a medicinal plant in Mexico and elsewhere in the world [10]. The avocado seed represents 13–18% of the fruit, and it is a byproduct generally not utilized. Normally, the seed is discarded during the processing of the pulp. The seed waste may represent a severe ecological problem [11]. But it may be of interest to industry as a source of bioactive compounds. It is reported to contain phytosterols, triterpenes, fatty acids, and two new glucosides of abscisic acid [12]. Several biological activities of the avocado seed have been reported such as antioxidant, antihypertensive, larvicidal, fungicidal, hypolipidemic, and recently amoebicidal and giardicidal activities [13]. Additionally, several studies have focused on the evaluation of acute toxicity of the fruit and leaves [14].

The seed of *Persea americana* has a diverse application in ethnomedicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites to the area of skin treatment and beautification [15]. The seeds are rich in tannins and carotenoids and tocopherols from the fruit were shown to inhibit the in-vitro growth of prostate cancer cell lines [16].

Currently, the seed represents an under-utilized resource and a waste issue for avocado processors. The avocado seed is discarded in the majority of countries, although in

some countries such as Niger, it is consumed [17,18].

Alhassan and colleagues evaluated the hypoglycaemic activity of *P. americana* aqueous seed extracts on alloxan-induced diabetic rats and concluded that the anti-diabetic effects of the extract might be due to certain mineral elements and phytochemicals and that an increase in weight could be due to proper nutrient utilisation that is most likely induced by the avocado seed extract [19]. However, the work by Okonta et al. [20] suggests that *P. americana* can lower blood glucose levels in cases of mild hyperglycemia but not severe hyperglycemia. Edem et al. [21] studied the effects of aqueous alligator pear seed extracts on normal and alloxan-induced diabetic rats, and their results suggested a restorative (or protective) effect of the extract on pancreatic islet cells.

The work of Mahadeva et al. [22], concentrated on the mechanism of the anti-diabetic activity of *P. americana*. The insulin-stimulative and antioxidative effects of *Persea americana* were evaluated in streptozotocin (STZ)-treated rats. This group found that the activities of pathophysiological enzymes such as serum aspartate transaminase (AST), serum alanine transaminase (ALT), and serum alkaline phosphatase (ALP) were altered in the serum of rats that had been treated with glyclazide, which was used as the standard reference drug, but not control rats. These results revealed the tissueprotective nature of *Persea americana* fruits [22].

The aforementioned studies provided further insight into the restorative and antioxidant activities of *P. americana*. However, the tissue eprotective potential of *P. americana* necessitated a look into the histopathological activity of the plant extract in the pancreas, liver and kidneys. Therefore, this study was conceived and designed based on the obvious gaps in what had been done or was known about this plant.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Samples of ripe avocado pear (*P. amercana*) were purchased from the New market, Enugu. The succulent fleshy part of the fruit was removed to obtain the seed. The seeds were minced by means of a grater and dried to a constant weight in an oven at 50°C. It was then ground to powder and then stored in a container. 100 g of the sample was extracted with 1000 ml of water and methanol. This was achieved using the maceration method. 100 g of dried, ground sample materials were soaked in water (100%) and methanol (1L of 80% methanol in 20% water) for 5 - 7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated under vacuum using a rotary evaporator at 40°C and stored at 4°C until when needed.

2.2 Animal Experiment

Twenty-eight (28) apparently healthy male rats of body weights ranging between 160 g-220 g were used for the experiment. The rats were housed in group of four in a photoperiod cycle of 12h:12h (Light and dark), at room temperature (28°C) and fed with standard laboratory diet and distilled water for a period of one week for acclimatization. Groups 2, 3, 4, 5, 6 and 7: were induced with diabetes by the intraperitonal (IP) injection of 150 mg/kg body weight of alloxan monohydrate solution. The rats were assigned into seven (7) groups of four (4) rats per group as shown below;

Group I- Normal untreated rats (negative control)

Group II- Diabetic untreated rats (positive control)

Group III- Diabetic rats treated with insulin (1unit of u40/50 g b.w./day).

Group IV-Diabetic rats treated 200mg/body kg water extract.

Group V- Diabetic rats treated with 300 mg/body kg water extract.

Group VI- Diabetic rats treated with 200 mg/body kg methanol extract.

Group VII- Diabetic rats treated with 300mg/ body kg methanol extract.

2.3 Induction of Diabetes

The baseline blood glucose levels of the rats were determined before they were induced with diabetes by intraperitonal (IP) injection of 150 mg/kg body weight of alloxan monohydrate

solution [23]. The blood glucose levels of the animals were determined using a Tyson Bio Evolve glucometer. The blood glucose levels of the rats were determined on a weekly basis for three (3) weeks of administration of the extracts. The body weights of the rats before induction, after induction and at intervals during the extract administration were noted. The treatment was withdrawn after a 21 days regime.

2.4 Administration of Extracts

The prescribed doses of plant extracts were orally administered to the rats daily, for 21 days of experiment.

2.5 Blood Sample Collection

Blood sample collection was done using the periorbital method. The animal was scuffed with thumb and the forefinger of the non-dominant hand and the skin around the eye was pulled taut. A capillary was inserted into the medial canthus of the eye (30 degree angle to the nose). Slight thumb pressure was enough to puncture the tissue and enter the plexus/sinus. Once broken, blood collection takes place. As soon as the required volume of blood was collected from plexus, the capillary tube was gently removed and wiped with sterile cotton. Bleeding was stopped by applying gentle finger pressure.

2.6 Liver Function Tests

All they tests below were determined using the colorimetric, end point method as described by Kochmar and Moss [24].

3. RESULTS

3.1 Glucose Analysis Results

The results of the glucose analysis as observed on a weekly basis are as shown in the Table 1.

3.2 Results of Body Weight Analysis

The results of the body weights of the rats were collected and analyzed as observed on a weekly basis are as shown in the Table 2.

3.3 Liver Function Test Results

The results of the various liver function parameters of the rats were collected and analyzed at the end of the experiment are as shown in the Table 3.

4. DISCUSSION

Table 1 show that there is no significant difference ($p > 0.05$) in the glucose levels in all the groups before diabetes was induced. This implies that the blood Glucose level in all groups before induction of diabetes is comparable.

The blood glucose level at day 1 (Table 1) shows that there is no significant difference in the mean glucose levels ($p > 0.05$) between Group I and Group II (5.39 mmol/L and 9.69 mmol/L). However, Group 3 (9.69 mmol/L) is significantly higher above the normal glucose level (3.7 mmol/L—5.6 mmol/L) indicating manifestation of diabetes. There is no significant difference ($p > 0.05$) between the other Groups V, VII, IV, VI and II (28.47 ± 4.92 mmol/L, 24.27 ± 5.31 mmol/L, 22.07 ± 6.45 mmol/L, 21.60±10.18 mmol/L, and 16.93 ± 2.82 mmol/L) respectively with Group IV having the highest glucose level (28.47±4.92 mmol/L) and it follows the trend with Group II having the lowest glucose level (16.93±2.82 mmol/L). However Groups V, VII, IV, VI and II are significantly different ($p < 0.05$) from the Group I and Group III. This shows that the extract treatment had not taken any significant effect as at day 1.

At day 7 as shown in Table 3 there is no significant difference ($p < 0.05$) between Group I and III and Group VI, (5.35±0.88 mmol/L, 8.92±2.36 mmol/L and 7.45±0.87 mmol/L) the implication of this is that Group VI (7.45± 0.87 mmol/L) significantly lowered the blood glucose to a level comparable to that of the control Group I (5.35± 0.88 mmol/L). In Group VI also, there was a significant percentage decrease in blood glucose (65.5%), meaning that Group VI (200mg/kg. b.wt. methanol extract) treatment has the potential to modulate high blood glucose.

Glucose levels of groups IV, V and VII (15.53±1.12 mmol/L, 14.32±2.62 mmol/L and 13.90±1.51 mmol/L) respectively are not significantly different ($p > 0.05$) from the untreated diabetic Group II (17.49 ± 6.52 mmol/L). Therefore, at day 7, the best treatment is Group VI (200 mg/kg. b.wt. methanol extract) which presented the lowest blood Glucose level (7.45±0.87 mmol/L) representing a 65.5% decrease.

At day 14 of the treatment as shown in Table 1, there is no significant difference ($p > 0.05$) between Groups IV, VI and I (7.36±2.45 mmol/L, 6.13±1.14 mmol/L and 4.31±0.55 mmol/L)

showing that treatments IV and VI gave similar blood glucose level and positively modulated the effect of the high blood glucose after day 14.

Table 1. Effect of Extracts on Blood glucose levels (mmol/L) of normal and diabetic rats.

Group	Day 0	Day 1	Day 7	Day 14	Day 21
I	4.47±0.52^{a}	5.39±0.42^{a}	5.35± 0.88^{a}	4.31±0.55^{a}	5.71±1.31^{a}
II	3.78±0.34^{a}	16.93±2.82^{b}	17.49 ± 6.52^{b}	24.11±6.37^{e}	24.13±7.36^{c}
III	3.94±0.69^{a}	9.69±2.75^{a}	8.92± 2.36^{a} (↓8.0)	10.55±1.28^{bc}(↑8.9)	8.27±2.36^{a} (↓14.7)
IV	5.22±1.74^{a}	22.07±6.45^{b}	15.53±1.12^{b}(↓29.6)	7.36±2.45^{ab}(↓66.9)	11.09±1.78^{ab}(↓49.8)
V	4.26±1.35^{a}	28.47±4.92^{b}	14.32±2.62^{b} (↓49.7)	12.83±3.46^{cd}(↓49.7)	15.17±3.12^{b}(↓46.7)
VI	4.27±0.00^{a}	21.60±10.18^{b}	7.45± 0.87^{a} (↓65.5)	6.13±1.14^{ab}(↓71.6)	6.34±0.59^{a}(↓70.7)
VII	3.94±0.53^{a}	24.27±5.31^{b}	13.90±1.51^{b} (↓42.7)	16.44±1.39^{d}(↓32.3)	9.99±1.73^{a}(↓58.8)

Mean ± S.D. Means with different superscript in the same column are significantly different (p<0.05).Figures in parenthesis indicates percentage decrease↓ or increase↑ in blood glucose level when compared to normal blood glucose levels.
Key; I = Normal Rats;
II = Diabetic untreated group;
III = Insulin treated group;
IV = 200 mg/ b.wt. water extract treated group;
V = 300 mg/ b.wt. water extract treated group;
VI= 200 mg/ b.wt. methanol extract treated group;
VII = 300 mg/ b.wt. methanol extract treated group.

Table 2. Effect of Extracts on Mean Bodyweight (g) of normal and diabetic rats.

Group	Initial weight	Day 7	Day 14	Day 21
I	155.43 ±11.30^{a}	171.23±16.68*(↑10.2)	198.93±28.02^{b}(↑16.2)	214.48±30.15*(↑7.9)
II	208.58 ±21.63^{b}	195.63±19.01*(↓6.2)	180.43±11.45^{ab}(↓7.8)	177.33±19.10*(↓1.7)
III	166.18 ±14.28^{a}	184.03±22.31*(↑10.7)	189.18±22.49^{ab}(↑2.8)	205.30±19.69*(↑8.5)
IV	157.35 ±33.98^{a}	154.08±41.43*(↓2.1)	159.95±27.63^{a}(↑3.8)	165.33±26.98*(↑3.4)
V	217.68 ±28.43^{b}	207.30±24.18*(↓4.8)	209.780±23.63^{b}(↑1.2)	212.70±22.14*(↑1.4)
VI	171.73 ± 5.60^{a}	178.33±45.37*(↑3.8)	200.90±20.90^{b}(↑12.7)	210.83±28.94*(↑5.0)
VII	152.65 ±13.80^{a}	149.85±19.42*(↓1.8)	149.90±19.09^{a}(↑0.03)	155.50±20.37*(↑3.7)

*Mean ± SD. *Not significant, Means with the same superscript in the same column are not significantly different (p < 0.05). Figures in parenthesis indicate percentage decrease↓ or increase↑ in body weights.*
KEY; I = Normal Rats;
II = Diabetic untreated group;
III = Insulin treated group;
IV = 200 mg/ b.wt. water extract treated group;
V = 300 mg/ b.wt. water extract treated group;
VI= 200 mg/ b.wt. methanol extract treated group;
VII = 300 mg/ b.wt. methanol extract treated group.

Table 3. Effect of Extracts on Mean Liver Parameters of normal and diabetic rats.

Group	Total Bilirubin (mg/dl)	Conjugate Bilirubin (mg/dl)	Aspartate transaminase (IU/L)	Alanine transaminase (IU/L)	Alkaline phosphatase (IU/L)
I	0.7±0.03^{a}	0.31±0.19^{a}	33.75±5.50^{a}	54.75±17.33^{a}	30.50±3.32^{a}
II	2.93±0.7^{bc}	1.38±0.21^{bc}	57.00±16.91^{c}	101.00 ±24.90^{b}	167.25±24.24^{d}
III	0.68±0.24^{a}	0.28±0.10^{a}	33.50±11.68^{a}	58.00±24.43^{a}	27.50±3.32^{a}
IV	1.85±0.8^{b}	1.20±0.53^{b}	39.00±4.11^{a}	52.50±12.07^{a}	58.25±8.18^{c}
V	2.88±0.1^{bc}	1.73±0.36^{c}	49.50±2.08^{bc}	52.25±5.32^{a}	52.00±8.68^{bc}
VI	3.75±0.26^{c}	2.23±0.51^{d}	44.25±3.30^{ab}	46.25±13.40^{a}	52.25±11.70^{bc}
VII	5.78±0.8^{d}	4.65±0.06^{e}	31.75 ±1.71^{a}	34.50±4.43^{a}	37.25±1.71^{ab}
Normal Range	**0.2—1.0**	**0.05—0.5**	**10— 40**	**5— 35**	**9 — 35**

Values are mean ± standard deviation, n=4. Means with the same superscript in the same column are not significantly different (p < 0.05).
KEY; I = Normal Rats;
II = Diabetic untreated group;
III = Insulin treated group;
IV = 200 mg/ b.wt. water extract treated group;
V = 300 mg/ b.wt. water extract treated group;
VI= 200 mg/ b.wt. methanol extract treated group;
VII = 300 mg/ b.wt. methanol extract treated group.

Also, no significant difference ($p > 0.05$) in glucose levels exist between group III, IV and VI (10.55±1.28 mmol/L, 7.36±2.45 mmol/L and 6.13±1.14 mmol/L respectively). These doses of the seed extracts could be effective as insulin in the control of blood sugar. There is no significant difference ($p > 0.05$) between Group III and Group V which also suggest the treatment in Group IV that could marginally lower blood glucose. No significant differences ($p > 0.05$) between Group V and VII (12.83±3.46 mmol/L and 16.44±1.39 mmol/L respectively) therefore, both have little effect in modulating the effect on high blood glucose. There is significant difference ($p < 0.05$) between all the Groups and Group II (24.116.37 mmol/L) showing that all the treatments have effects in modulating the effect of high blood glucose.

At the end of 21 days of treatment (Table 1), there is no significant difference ($p > 0.05$) between Groups I, VI, III, VII, (5.71±1.31 mmol/L, 6.34±0.59 mmol/L, 8.27±2.36 mmol/L, 9.99±1.73 mmol/L), and IV and (11.09 ± 1.78 mmol/L). This suggests that treatment Groups VI and VII (methanol extract treated with 200 and 300 mg/kg. b.wt. respectively) may lower effectively, and Group IV (300 mg/kg.b.wt) may marginally substitute for insulin in lowering high blood glucose back to normal Control Group level (5.71±1.31 mmol/L) in the long run. No significant difference ($p > 0.05$) exists between group IV and V (11.09±1.98 mmol/L and 15.17±3.12 mmol/L) respectively, which indicates that they have similar effect, with Group IV (11.09±1.98) having a better blood glucose lowering effect. No significant difference ($p > 0.05$) exists between group II and all the other groups showing that group two is diabetic while others modulated the effect to varying degree.

As shown in the Table above, methanolic extract treated Groups VI and VII showed higher decrease in their glucose levels than the water extract treated Groups IV and V. Among the induced diabetic rat Groups, rats treated with the seed extracts presented a higher percentage decrease in blood glucose levels than those treated with insulin.

Results from the present study showed that the groups treated with methanol seed extracts showed a better result than the water seed extracts in terms of its anti-diabetic effect. The extracts were able to lower the blood glucose of the rats even better than insulin which is an established anti-diabetic agent. In general, Group VI (200 mg/kg b.wt. methanol extract dose Group) showed the best performance in terms of modulation of high blood glucose.

The hypoglycemic effect of avocado seed observed in the present study agrees with results of earlier studies. Ezejiofor et al. [25] reported a percentage reduction of blood glucose of diabetic rats treated with aqueous extracts of avocado seed ranging from 45.8% (200 mg/kg on day 7) to 58.9% (400 mg/kg on day 21). Similarly, Alhassan et al. [19], reported a significant decrease in the blood glucose level of diabetic rats treated with water extract of avocado seed. N'guessan et al. [2] reported a dose of 30 g/L decoction of aqueous avocado seed extract effectively restored the blood glucose levels of diabetic rabbits to its normal level. Edem, [26] observed a 73.26% and 78.24% decrease of blood glucose of diabetic rats treated with 300 mg/kg and 600 mg/kg body wt respectively of aqueous avocado pear seed extract. The anti-diabetic effects of avocado plant is not only restricted to its seed. Various studies have revealed that leaves of avocado plant also possess anti diabetic properties [27,28].

The anti-diabetic effects of avocado seed extracts indicate the presence of hypoglycaemic agents in the *P. americana* seed. Hypoglycemic effect of the avocado seed extract may probably be due to contents of elements such as calcium, magnesium, potassium, sodium, zinc, chromium [29]. These elements play role in blood glucose homeostasis by regulating the key enzymes involved in gluconeogenesis in the liver e.g. glucose-6- phosphatase, fruitcose-1, 6-bisphosphatatase and phosphoenolpyruvate carboxykinase, thereby blocking gluconeogenesis and enhancing glucose utilization in the body [29]. The seed may contain certain hypoglycemic agents such as phytochemicals like tannins. It might also contain insulin stimulatory substances such as insulin receptors substrate (IRS), glycogen synthase, the b3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor [29]. However, the mechanism by which the extract lowered the blood glucose level in alloxan induced diabetic rats is still unclear. It could be by stimulating peripheral utilization of glucose by inhibiting absorption in the gastrointestinal tract (GIT), increasing glucose metabolism, or regenerating the pancreatic tissue or potentiating the insulin secretion by the surviving B- cells [19].

Furthermore, tannins were reported by Tiwari and Rao, [30], to possess hypoglycaemic

properties via an inhibitory action on the sodium-glucose transporter 1 (S-GLUT1). Dietary intake of tannins might prove to be important for alternative diabetes treatments or reduction of the risk of the disease [31]. Attempts have been made to determine their potential in preventing β-cell apoptosis, promoting β-cell proliferation and insulin secretion [32], and enhancing insulin activity [33].

Results from several studies have also shown that diabetes can be managed by herbal approaches with results comparable with the result obtained in the present study. Plants such as *Gallega officinalis* [34-36] *Syzygium cumini* [37,38], are reported to have anti diabetic properties. Interestingly, these studies were carried out with different parts of the plants such as seed, leaves and even flower. Anti hyperglycemic activity of *Allium sativum* (garlic) was reported to be most potent when administered at 0.25 mg/kg b.wt dose level which was due to increased insulin-like activity (Ayodhya et al., 2010). Oral administration of the juice, ethanol extract, and oil of *A. sativum* has remarkably blood sugar lowering effect in normal and alloxan-induced diabetic rats or rabbit suggesting stimulation of insulin secretion from parital cells of pancreas [39]. About 94% seed diet of *Acacia arabica* showed hypoglycemic effect in rats through release of insulin. *Acacia arabica* seed powder at 2, 3 and 4g/kg. b.wt., exerted a significant hypoglycemic effect in normal rabbits by stimulating the release of insulin from pancreatic beta cells [40].

As shown in Table 2, results of the body weights showed that as at day 7, no significant difference ($p > 0.05$) exists between the body weights of the groups. After one week of diabetes and treatment, it was observed that with the exception of Groups I, III, and VI, (Normal Control, insulin and 200mg/kg b.wt. methanol extract treated respectively), all gained weight, while the other Groups lost weight. This weight loss could be attributed to the action of diabetes. The ability of alloxan induced diabetes to induce weight loss in diabetic untreated rats mimics what is commonly observed in clinical diabetes [41]. The insulin treated and 200 mg/kg. b.wt. methanol extracts doses, (Groups III and IV) showed little weight gain. This suggests a possible short-term positive effect of group IV on body weight of diabetic rats.

After 14 days of treatment, there was no significant difference ($p > 0.05$) between the mean weights of groups (Table 2). Results also showed that the treated Groups (III, IV, V, VI and VII) gained weight. Indicating that probably both insulin and the extracts (both water and methanol) stimulated weight gain. However, the untreated diabetic control group (II) continued to lose weight due to the effect of diabetes. Highest percentage weight gain (12.7%) was recorded for Group VI. The lowest weight gain occurred in Group VII (0.03%). The weight gain in Group VI (12.7%) is higher than that recorded for the insulin treated Group III (2.8%) but on the same level with that of the Normal control Group I (16.2%).

However, at day 21, results of all the groups showed no significant difference in their body weight ($p > 0.05$) between them. The Groups treated with insulin, and extracts (Groups III, IV, V, VI, and VII) also continued its weight gain which is also a trend within the Normal Control Group. After 21 days, untreated Group (Group II) lost weight as with the case with diabetes.

There is a significant between in weight gain between the water and methanolic seed extracts Groups (IV, V, VI and VII) and untreated Group (Group II). Weight gain observed in Groups treated with the extract shows that avocado seed extracts could be useful for the management of weight loss attributable to diabetes. Group VI presented the best result by increasing weight gain by 21.5% over the 21 days treatment regime. The restoration of body weight by 21.5% activity of *P. americana* seems to be due to its lowering blood sugar property by increased glucose metabolism, and this may be due to the protective effect of the extract in controlling muscle wasting, by reversal of gluconeogenesis [25].

From Table 3, analysis of the Liver enzymes of this study indicates that alloxan induced diabetes caused elevated levels of liver enzymes in rats. This observation is consistent with previous reports by Felig et al. [42], and Iweala and Oludare, [43]. Results of liver function test showed a significantly ($p < 0.05$) increased liver enzyme levels in the untreated diabetic rats as compared with the treated groups (insulin and extracts treated groups). This could be due to possible liver damaging effect of alloxan induced diabetes. This alloxan liver damaging effect was also reorted by earlier studies [44-46].

Results from Table 3 shows the bilirubin levels of experimental rats. There is significant difference

($p < 0.05$) in the total bilirubin levels between Groups I and III (0.7±0.03 mg/dl and 0.68±0.24 mg/dl respectively). Bilirubin levels of Groups I and III were significantly lower than those of Groups IV, V, II, VI, and VII. It seems insulin treatment may have lowered bilirubin level. The bilirubin levels of Groups IV, II and V (1.85±0.5 mg/dl, 2.93±0.03 mg/dl and 2.88±0.1mg/dl) are not significantly different ($p > 0.05$) from each other. Bilirubin levels of Groups II, V and VI (2.93±0.03 mg/dl, 2.88±0.1 mg/dl and 3.75±0.26 mg/dl respectively) are not significantly different ($p > 0.05$) from each other. Group VII (5.78±0.8 mg/dl) recorded a significantly ($p < 0.05$) higher total bilirubin than the other groups. It appears methanol extract significantly elevate total bilirubin level compared to the normal control and insulin treated. Among the extract treated groups, Group IV (200 mg/kg.b.wt. water extract treated dose) is significantly lower in total bilirubin than group V, VI and VII. However, only groups I and II were within normal bilirubin level range (0.2 mg/dl—1.0 mg/dl). The other groups (II, IV, V, VI and VII) have higher bilirubin levels than the normal range, suggesting a possible increase in total bilirubin due to either the effect of diabetes or the various plants extracts. This study shows that there could be a relationship between bilirubin level and the induction or incidence and management of diabetes. It shows that diabetes can lead to elevated levels of bilirubin. It is reported that while low serum bilirubin concentrations are associated with an increased risk of diabetes; mildly elevated serum bilirubin levels provide protection against diabetes [47]. Another study on high total bilirubin as a protective factor for diabetes mellitus concluded that higher levels of total bilirubin increase glucose mobilization into the cells, leading to more efficient, biologic glucose utilization [48].

Results of conjugate bilirubin, (Table 3) shows there is no significant difference ($p > 0.05$) between Groups I and III (0.31±0.19 mg/dl and 0.28±0.10 mg/dl respectively). There was no significant difference ($p > 0.05$) in the conjugate bilirubin levels between Groups I and II (1.38±021 mg/dl and 1.20±0.53 mg/dl respectively), they also have comparable conjugate bilirubin level. There was also no significant difference ($p > 0.05$) between Groups II and IV (0.31±0.19 mg/dl and 1.73±0.36 mg/dl resectively) they also have comparable conjugate bilirubin level. This means that both water extract groups compared with the untreated diabetic group. However, Group VI has a conjugate bilirubin (2.23±0.51mg/dl) which is significantly ($p < 0.05$) higher than Groups I to V (0.31±0.19 mg/dl 1.38±0.21 mg/dl, 0.28±0.10 mg/dl, 1.20±0.53 mg/dl and 1.73±0.36 mg/dl) and significantly lower than Group VII. Group VII (4.65±0.06 mg/dl) is significantly higher than every other group. The methanolic extract Groups (VI and VII) has higher conjugate bilirubin levels (2.23±0.51 mg/dl and 4.65±0.06 mg/dl). This further show that methanolic extracts also significantly elevate conjugate bilirubin concentration compared to both the insulin and water extract treated groups. Groups I and III (0.31±0.19 mg/dl and 0.28±0.10 mg/dl), have conjugate bilirubin levels within the normal range level (0.0 mg/dl—0.5 mg/dl). While the other groups have their conjugate bilirubin levels above the normal range (0.0 mg/dl—0.5 mg/dl). Increase in conjugate bilirubin level was also reported by Chome et al. [49]. who concluded that 62% of the circulating bilirubin was conjugated in the diabetics and only 25% in the non-diabetics.

AST levels as shown in Table 3, indicates that no significant difference ($p > 0.05$) between groups VII, III, I, IV, and VI (31.75±1.71 IU/L, 33.50±11.68 IU/L, 33.75±5.50 IU/L, 39.00±4.11 IU/L and 44.25±3.30 IU/L respectively). On comparison, the normal Group (Group I) and Group VI (300 mg/kg. b.wt. methanol treated) have a lower AST level than the other treated groups (water and methanol extracts treated groups), however, this difference is not significant ($p > 0.05$) Within diabetic treated groups (III, IV, V, VI and VII) Group VII (300 mg/kg. b.wt. methanol treated) has a lower AST level than both the insulin and extracts (water and methanol) treated groups, but there is no significant difference ($p > 0.05$) between the groups. There is no significant difference ($p > 0.05$) between groups V and II (49.50±2.08 IU/L and 57.00±16.91 IU/L). Within the extract treated groups, Groups IV and VII (39.00±4.11IU/L and 31.75 ±1.71 IU/L) showed the best result by presenting the lowest levels of aspartate transaminase. However, on comparison with the normal AST levels (10IU/L—40IU/L), Groups I, III, IV and VII all presented AST levels within the normal range (10IU/L—40IU/L); Groups II, V, and VI showed an increase in AST level. However, group II (57.00±16.91IU/L) showed the highest level of AST, implying that there could be a relationship between diabetes and AST levels. This is also an indication of liver injury associated with alloxan induced-diabetes.

ALT levels (Table 3) reveals there is no significant difference ($p > 0.05$) between groups III, I, IV, V, VI and VII (58.00±24.43IU/L, 54.75±17.33 IU/L, 52.50±12.07IU/L, 52.25±5.32 IU/L, 46.25±13.40 IU/L, and 34.50±4.43 IU/L), however Group I has the lowest ALT levels. Therefore, each treatment appears to modulate the activity of Alanine transaminase. Alanine transaminase level in Group II (101.00 ±24.90 IU/L) presented a higher value than those of the other Groups implying that high blood glucose (diabetes) may affect liver activity. Comparing with the normal ALT range, (5IU/L—35IU/L), Group VII (34.50±4.43 IU/L) presented a value within the normal range while all the other Groups have values above the normal range. It is assumed that elevation in the activities of ALT, AST, and ALP are considered predictors of diabetes mellitus. Furthermore, elevation in levels of these gluconeogenic enzymes, whose gene transcription is suppressed by insulin, could indicate impairment in insulin signaling rather than purely liver cell injury (O'Brien and Granner, 1991).

The ALP levels (Table 3) showed that there is no significant difference ($p > 0.05$) between Group III, I and VII (27.50±3.32 IU/L, 30.50±3.32IU/L and 37.25±1.71IU/L) with Group III (insulin treated group) having the lowest enzyme level. There is no significant difference ($p > 0.05$) between Groups VII, V, and VI (37.25±1.71IU/L, 52.00±8.68IU/L and 52.25±11.70 IU/L). There is no significant difference ($p > 0.05$) between Groups V, VI and IV (52.00±8.68 IU/L, 52.25±11.70 IU/L and 58.25±8.18IU/L). Group II (167.25±24.24IU/L) is significantly ($p < 0.05$) higher than every other group also highlighting the effect of diabetics on liver function parameters. Comparing with the normal ALP range (9IU/L—35IU/L), Groups III, I and VII (27.50±3.32IU/L, 30.50±3.32IU/L and 37.25±1.71IU/L) all presented values within the normal. Groups V, VI and IV (52.00±8.68IU/L, 52.25±11.70IU/L and 58.25±8.18IU/L) presented values that were mildly higher than the normal range.

All the treatments modulated the liver enzymes in varying proportions. Group VII appears to have the best result by showing liver function parameter levels that are within their various, followed closely by Groups VI and V. The methanolic extract treated Groups (VI and VII) were observed to significantly ($p > 0.05$) elevates the total bilirubin and conjugate bilirubin concentration. Group IV showed positive effect on some liver function parameters like total bilirubin, aspartate transaminase and alanine transaminase.

Thus, the increased activities of ALT, AST and ALP in serum of diabetic rats may primarily be due to leakage of these enzymes from liver cytosol into the blood stream as a consequence of hepatotoxic effect of alloxan [50].

However, the relatively higher values of the groups treated with the seed extracts show that there may be presence of certain toxic compounds in the extracts. Similar toxic effects of *Persea americana* and its extracts was reported by Trinder, [51]; and Friday *et al.*, [52]. Also it has been reported that the administration of the liquid extract continuously could cause a liver damage and increase in plasma enzymes AST and ALT [52].

Studies by Imafidon, [53] also reported on the liver enzyme lowering properties of avocado seed. Acute and sub-acute toxicological assessment of the aqueous seed extract of *Persea Americana* Mill (Lauraceae) in Rats reported by Ozolua, et al. [54] showed lower levels of liver enzymes compared to normal. Oyeyemi and Oyeyemi, [55] reported similar results, indicating lower liver enzymes in avocado treated rats, while rats treated with *Parinari polyandra* seed extract showed decrease in levels of ALT and AST [43,56]. Abbas and Qureshi, [45] also reported lower levels of liver enzymes in alloxan induced diabetic rats treated with *Woodfordia fruticosa* Linn.

5. CONCLUSION

Results from this study indicate indicates that water and methanol extracts of *P. americana* seed exerts significant anti-diabetic property in rats. It also presented a tissue repair effect on both the kidney and liver following tissue damage by alloxan. These observations provide a pharmacological basis for the traditional use of avocado seed in the management of diabetes mellitus. However, further studies are required to identify the active ingredient responsible for the anti-diabetic properties of the seed extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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