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Research Article

Antidiabetic and hypolipidaemic potentials of ethanol fruit pulp extract of *Persea americana* (avocado pear) in rats

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ABSTRACT

Background: Development of new therapies capable of improving glycaemia and lipid profiles in diabetes management without side effects, reduction in efficacy and toxicity has been of great scientific interest. *P. americana* seed has been reported to have diverse applications in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin treatment and beautification however there is dearth of information regarding it use in treatment of diabetes and hypolipidemic effect following its use for other purposes. **Aim**: Therefore, this study was undertaken to investigate the antidiabetic and hypolipidaemic potentials of *P. americana* ethanol fruit pulp extract. **Methods:** Phytochemical screening for classes of secondary plant metabolites was done using standard methods. 250 and 500mg/kg of *P. americana* ethanol fruit pulp extracts were administered to alloxan- induced diabetic rats orally twice daily for 3 weeks. Glycemic levels were checked every 3 days and serum lipid profile assay was carried out at the end of the treatment period. **Results:** Phytochemical screening of the extract revealed presence of various classes of phytochemicals such as saponins, tannins, alkaloids and steroids**.** Both doses of the extract significantly reduced blood glucose levels when compared to the control group. The higher dose (500mg/kg) significantly decreased total cholesterol, triglycerides and low-density lipoproteins compared with the control. There was also a marginal increase in HDL-Cholesterol. **Conclusion:** *P. americana* ethanol fruit pulp extract reduces hyperglycaemia and hyperlipidaemia associated with type I diabetes mellitus.

INTRODUCTION

Persea americana fruit tree originated in South Central Mexico (Royal Botanic Gardens, Kew and Missouri Botanical Garden 2010; Chen *et al*., 2008). It is classified as a member of the flowering plant family *Lauraceae*, the fruit of the plant also called avocado pear or alligator pear, is botanically a large berry containing a single large seed known as a "pit" or a "stone" (Morton, 1987; Storey, 1973). The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and of smooth, almost creamy texture (Morton, 1987). *P. americana* leaves have been reported to have anti-inflammatory and analgesic activities (Adeyemi *et al*., 2002). The seed of *P. americana* has diverse applications in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin diseases and beautification (Pamplora and Roger, 1999). Antioxidant activity due to the phenolic content of seeds of avocado pear was found to be greater than 70% (Song and Barlow, 2004). Avocados are one of the few fruits that give “good” fats because it contains lipids such as phytosterols, ß-sitosterol, campesterol, and stigmasterol as well as monounsaturated fatty acids mainly oleic acid and it also reduces the risk of cardiovascular disease (Olagunju *et al*., 2017).

Diabetes mellitus is a complex metabolic disorder that mainly occurs due to defects in either insulin secretion, insulin action, or both and is characterized by high blood sugar (glucose) levels **(**Kooti *et al*., 2016**)**. The disorder can also lead to serious complications affecting human health with long-term effects that causes micro and macro vascular problems (Mohana *et*

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al., 2012). The World Health Organization reports suggests that the prevalence of diabetes in adults worldwide would increase to 300 million in years 2025, making the disease one of the main threats to human health in the 21st century and also the fifth leading cause of deaths in most developed countries (Kazi, 2014).

Type 1 diabetes mellitus or insulin dependent diabetes mellitus (IDDM) involves ß-cell destruction with little or no endogenous insulin secretory capacity and is triggered by autoimmune idiopathic factors (Bastaki, 2005). A major feature of Type 2 diabetes mellitus is insulin resistance or deficiency, which can cause hyperglycemia (Laakso, 2001). High prevalence, variable pathogenesis, progressive process, and complications of diabetes all highlight the urgent need for effective treatments such as insulin therapy, pharmacotherapy, and diet therapy **(**Kooti *et al*., 2016**)**.

Despite the significant progress made in the treatment of diabetes, treatment outcomes are are still far from perfect due to drug resistance (reduction of efficacy), side effects, and toxicity (Hui *et al.,* 2005). However, the use of medicinal plants is now being recommended because most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides which often have anti-diabetic effects (Michael *et al*., 2005; Kooti *et al*., 2015). The aim of this study was to investigate the antidiabetic and hypolipidaemic potentials of ethanol fruit pulp extract of *P. americana* on alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of Fruits pulp extract

Ripe fruits of *P. americana* were obtained from Enugu, and were authenticated in Botanical unit, of the Department of Biological Sciences, Madonna University Nigeria. The fruits were thoroughly washed and the pericarp (peel) removed alongside the seeds from the mesocarp (pulp). The fruit pulps (Mesocarp) were cut into smaller pieces and air dried for 4 days at ambient temperature and thereafter grounded into powdered form using mortar and pestle. The powdered sample was extracted with ethanol using Soxhlet apparatus, concentrated to dryness in a water bath and preserved at 4ºC until required for use (Redfern *et al*., 2014). Weighed samples of the extract (1g in 10 ml distilled water) were then used to prepare the stock solution (100 mg/ml).

The brand of metformin (METFORMINA® 500 mg) used in this study was Manufactured by S Kant Healthcare Ltd. India. The Alloxan monohydrate (Sigma St. Louis, M.O., USA) solution was prepared by dissolving 1.25g of Alloxan monohydrate in 25ml of distilled water.

Experimental Animals and Treatment Protocol

Twenty-five male albino rats (160-200g) used for this experiment were obtained from the University Animal house and kept in standard rat cages, fed with pelletized commercial feed and tap water *ad libitum* followed by 1 week of habituation before the commencement of the research. Animal studies were carried out in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Research Council, 1999). The animals were then assigned into five groups of five animals each as shown below:

Group 1 – Normal Control (2 mg/kg of distilled water p.o., twice daily)

Group 2 – Diabetic Control

Group 3 – Diabetic + *P. americana* (250 mg/kg p.o., twice daily)

Group 4 – Diabetic + *P. americana* (500 mg/kg p.o., twice daily)

Group 5 – Diabetic + Metformin (500 mg/kg p.o., once daily)

Group's 2 – 5 animals were fasted overnight then diabetes was induced by a single intraperitoneal (IP) injection of freshly prepared 150 mg/kg of alloxan monohydrate solution (Yanarday and Colac, 1998). Animals were considered diabetic if the blood glucose values were ≥ 200mg/dl 48 hours after alloxan injection. Blood glucose levels were checked using a glucometer (Bioland glucometer, Germany).

Oral administration was done for 21 consecutive days with aid of a rubber cannula attached to a calibrated syringe. Blood glucose levels were checked every three days by 6am during the treatment period of 21 days by prickling the tail vein of the animals. The animals were fasted overnight on the 21st day, rendered unconscious under chloroform fumes, sacrificed and blood withdrawn via cardiac puncture was collected in plain-capped sample bottles. Blood samples collected were centrifuged at 3000rpm for 10 minutes. Then serum was separated gently and stored in labeled plain sample bottles at -20º C until required for the lipid profile assay.

Lipid Profile Analysis

Serum total cholesterol, triglyceride and high-density lipoprotein (HDL) were determined using Randox kits produced by Human Diagnostic-Germany (Schettler and Nussel, 1975; Trinder, 1981; Trinder and Webster, 1984). Serum very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated using the Friedewald's method (Friedwald, 1972).

Phytochemical Analysis of P. americana ethanol pulp Extract:
Using standard methods, the extract was screened for bioactive ingredients. Saponins and oxalates determined by the method of Kokate, (1997). Alkaloid, tannins and Phenols were determined by method of Trease and Evans (1989). Phytates, steroids and cardiac glycosides were determined by method of Harbone, (1973).

Statistical Analysis:
Data was analysed using one-way ANOVA and difference between groups compared using Least Significance Difference (LSD). Data are expressed as mean ± standard error of mean and values of $P<0.05$ were considered significant. SPSS version 15.0 was used for this analysis.

RESULTS

Effect of treatment on blood glucose levels of diabetic rats
Results in Table 1 shows the mean blood glucose concentrations of the control groups (normal, diabetic controls), the two doses of the *P. americana* ethanol fruit pulp extract and the standard drug (metformin).

Table 1: Effect of oral 21-day doses of *P. americana* ethanol fruit pulp extract on blood glucose levels of alloxan-induced diabetic rats.

Groups	Mean Blood glucose levels (mmol/l)	
	Initial	Final
Normal Control (2 mg/kg of distilled water p.o. twice daily)	6.70 ± 0.8	7.5 ± 0.6
Diabetic Control	16.65 ± 5.0*	29.3 ± 4.0*
Diabetic + *P. americana* (250 mg/kg)	20.10 ± 7.0*	14.4 ± 2.0bc
Diabetic + *P. americana* (500 mg/kg)	20.40 ± 5.0*	9.4 ± 1.0b
Diabetic + metformin (500 mg/kg)	25.20 ± 1.0*	6.7 ± 0.0b

Values are expressed in mean ± SEM, n = 4; * = $P<0.05$ indicates a significant difference compared with normal control; b = $P<0.05$ indicates a significant difference compared with diabetic control; c = $P<0.05$ indicates a significant difference compared with standard drug, metformin. Initial = 0 week after induction of diabetes and before commencement of treatment. Final = mean values obtained after 21 days of treatment.

Both doses of *P. Americana* ethanol fruit pulp extract (250 mg/kg and 500 mg/kg) significantly decreased ($P<0.05$) blood glucose levels in the diabetic treatment groups 3 (14.4 ± 2.0 mmol/l) and 4 (9.4 ± 1.0 mmol/l) respectively when compared to the diabetic control group (29.3 ± 4.0 mmol/l).
Also, in the alloxan-induced diabetic group treated with metformin (6.7 ± 0.0 mmol/l), a significant decrease ($p<0.05$) was observed in the blood glucose concentrations when compared to the diabetic control group (29.3±4.0 mmol/l).

Effect of treatment on serum lipid profiles of diabetic rats
Results obtained for serum lipid profiles showed significant ($P<0.05$) increases in total cholesterol, Triglycerides and low-density lipoproteins of the diabetic control group when compared to the normal control group and the Metformin group 4 (Table 2).
P. americana (500mg/kg) diabetic treatment group showed significant ($P<0.05$) decreases in TC, TG and LDL, when compared with the diabetic control. HDL-C also showed marginal increase in *P. americana* (500mg/kg) diabetic treatment group. (Table 2).

Table 2: Effect of oral 21-day doses of *P. americana* ethanol fruit pulp extract on serum lipid profiles of alloxan-induced diabetic rats.

Groups	Total cholesterol (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)
Normal control (2 mg/kg of distilled water p.o. twice daily)	4.2 ± 0.1	1.47 ± 1.1	1.9 ± 1.5	0.70± 0.1
Diabetic Control	11.3± 1.1*c	6.8± 0.4*c	1.4 ± 0.1	12.1 ± 0.4*c
Diabetic + *P. americana* (250 mg/kg)	6.4 ± 0.0bc	3.9 ± 0.4bc	2.1 ± 0.1	8.04 ± 0.1bc
Diabetic + *P. americana* (500 mg/kg)	2.5 ± 0.0b	1.9 ± 0.1b	2.8 ± 0.5	1.93 ± 0.4b
Diabetic + *Metformin* (500 mg/kg)	1.8 ± 0.0b	0.7 ± 0.3b	3.1 ± 0.2*b	0.63 ± 0.1b

TG; Triglycerides, HDL; high-density lipoproteins, LDL; low-density lipoproteins. Values are expressed in mean ± SEM, n = 4; * = $P<0.05$ indicates a significant difference compared with normal control; b = $P<0.05$ indicates a significant difference compared with diabetic control
c = $P<0.05$ indicates a significant difference compared with standard drug, metformin.

Phytochemical screening of ethanol pulp extract of Persea americana
The phytochemical analysis of the ethanolic pulp extract of *P. americana* revealed the presence of various concentrations of phytochemicals as shown in Table 3.

Table 3: Phytochemical constituent of ethanol pulp extract of *P. americana*

Constituent	Inference
Alkaloids	+
Saponins	+
Tannin	+
Phytate	+
Phenol	+
Cardiac-glycosides	+
Oxalate	+
Steroids	+

Key: + = present.

DISCUSSION

Uncontrolled diabetes can lead to serious micro and macro vascular problems on the long term (Mohana *et al*., 2012). In addition it causes many chronic complications including blindness, heart disease and renal failure (Mamun-or-Rashid *et al*., 2014).

Results obtained from phytochemical screening of the *P. americana* ethanol pulp extract showed it contains saponins, alkaloids, steroid, phytate, phenol, oxalate, tannins and glycosides, suggesting that they contribute to the therapeutic efficacy/anti-diabetic properties of the plant extract.

A significant change occurs in the structure and metabolism of lipid in diabetes leading to lipid peroxidation associated with hyperlipidaemia (Kooti *et al*., 2016). This finding was also observed in our study as the increased blood sugar was accompanied by alterations the lipid profiles of the untreated diabetic animals.

The study showed that daily oral administration of doses of ethanol fruit pulp extract of *P. americana* significantly reduced the blood glucose levels of the alloxan- induced diabetic rats close to normal values. These results are in line with findings by Alhassan *et al*. (2012) who reported that consumption of the aqueous seed extract of *P. americana* exerts significant hypoglycaemic effects on alloxan induced diabetic rats. The significant anti-diabetic activity of the ethanol fruit pulp extract of *P. americana* is due to the presence of hypoglycemic agents such as saponins, tannins, alkaloids and steroids which contain insulin stimulatory substances such as insulin receptors substrate (IRS), pro-hormone convertase, glycogen synthase, the beta-3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor and peroxisome proliferators (Broadhust, 1997).

Results of this study also showed the ability of *P. americana* ethanol fruit pulp extract to significantly reduce TC, TG, LDL while marginally (non-significantly) increasing HDL levels in diabetic treated animals. HDL has the ability to promote efflux of cholesterol from cells, which may minimize the accumulation of foam cells in the artery wall thereby preventing the development of atherosclerosis (Olagunju *et al*., 2017).

This research also supports findings by Olagunju *et al*. (2017) who reported that cardiovascular disease marker on TC/HDL ratio of male albino rats fed with aqueous and ethanol extracts of *P. americana* fell within the low risk acceptable range and boosting the "good cholesterol" (HDL) which is good for cardiovascular health. Therefore, the significant hypolipidaemic activity of *P. americana* ethanol fruit pulp extract in this study can be attributed to the presence of phytochemicals such as alkaloids, phenols, saponins and sterols in the extract (Bopanna *et al*., 1997; Katsumata, *et al*., 1999).

In conclusion, the administration of *P. americana* ethanol fruit pulp extract produced significant reduction of blood glucose levels and lipid related dysfunction in alloxan-induced diabetic rats. Thus, in the light of these studies, further pharmacological investigations are needed to determine the chemical compositions of the extract and their exact mechanisms of action in the management of diabetes.

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