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Research Article

Hypoglycaemic and hypolipidemic potentials of ethanol fruit pulp extract of *Persea americana* in alloxan induced diabetic rats

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ABSTRACT

Development of new therapies capable of improving glycaemia and abnormal lipid profiles in diabetes management without side effects, reduction in efficiency and toxicity has been of great scientific interest. Therefore, this study was undertaken to investigate the hypoglycemic and hypolipidemic potentials of *Persea americana* ethanolic extract. Phytochemical analysis was done using standard methods and extract screened for bioactive ingredients 250 and 500 mg/kg of P. americana ethanolic extract 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. Phytochemical screening of the extract revealed presence of various concentrations of phytochemicals, both doses of the extract significantly reduced blood glucose levels in the diabetic treatment groups when compared to the diabetic control group. High dose diabetic treatment group (500 mg/kg) showed significant decreases in total cholesterol, triglycerides and low-density lipoproteins compared with the lipid profile levels of the diabetic control accompanied by a marginal increase in HDL-C. *P. americana* extract produced desirable effects on hyperglycemia and hyperlipidemia associated with type I diabetes mellitus.

Keywords: *Persea americana*, Hyperglycemia, Diabetes mellitus, Serum lipid profile

Introduction

Persea americana fruit tree originated in South Central Mexico (Royal Botanic Gardens, Kew and Missouri Botanical Garden 2010; Chen et al., 2008). Classified as a member of the flowering plant family Lauraceae, the fruit of the plant also called an 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 flavoured, and of smooth, almost creamy texture (Morton, 2004). *P. americana* leaves have been reported to have or possess 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 treatment and beautification (Pamplora and Roger, 2004). Antioxidant activity and 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, Я-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 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 al., 2012). The World Health Organization reports suggests that the prevalence of diabetes in adults worldwide would

increase to 300 million in years 2025. It is the one of the main threats to human health in the 21st century and is the fifth leading cause of deaths in most developed countries (Kazi, 2014).

Type 1 diabetes mellitus or Insulin Dependent Diabetes Mellitus (IDDM) involves Я-cell destruction with little or no endogenous insulin secretory capacity 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). Also, there are several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms, which include stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing of peripheral absorption of glucose by biguanides and thiazolidinediones (Bathaie et al., 2012), delay in the absorption of carbohydrates from the intestine by alpha-glucosidase, and reduction of hepatic gluconeogenesis by biguanides (Pamplora and Roger, 1999).

Despite the significant progress made in the treatment of diabetes, the results of treatment in patients is still far from perfect and these treatments have some disadvantages, including drug resistance (reduction of efficiency), side effects, and even toxicity (Hui et al., 2015). However, many treatments that involve the use of medicinal plants are recommended because most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have anti-diabetic effects (Michael et al., 2005; Kooti et al., 2015). The anti-hyperglycemic effects that results from treatment with plants are often due to their ability to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose (Kooti et al., 2016). However, the aim of this study was to ascertain the antidiabetic and hypolipidemic potentials of ethanolic extract of *Persea americana* on alloxan induced diabetic rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of Fruits and Drugs

Ripe fruits of *Persea americana* were purchased from a local market in Kilombero District of Morogoro Region, Tanzania. The plant materials were authenticated at the Herbarium of the Department of Botany, University of Dar es Salaam, Dar es Salaam, Tanzania. The fruits thoroughly washed and the pericarp (peel) removed from the mesocarp (pulp). The pulp were cut into smaller pieces and air dried for 4 days at ambient temperature and thereafter grounded into powdered form using porcelain mortar and pestle. The powdered sample was extracted with ethanol using soxhlet apparatus, concentrated to dryness in a water bath and preserved at 4°C until required for use until required for use (Redfern et al., 2014). Weighed samples of the extract were then used to prepare the stock solution (100 mg/ml). The brand of Metformin drug used in the course of this research work had identification records as follows; METFORMINA 500 mg: Metformin tablets B.P. 500 mg is its composition. Manufactured by S KANT HEALTHCARE LTD. 1802-1805, GIDC. Phase-III, Vapi, Gujarat, India.

Experimental Animals and Treatment Protocol

Twenty-five male albino rats (160-200 g) were used for this experiment. They were housed in standard rat cages, fed with pelletized commercial feed (Hill animal feeds and Agrovet supplies Ltd, Bagamoyo-Pwani, Tanzania) and tap water ad libitum, then kept under laboratory conditions for an acclimatization period of seven days before the research commenced. The animals were assigned into five groups of five animals each as shown below;

Group 1-Normal Control

Group 2-Diabetic Control

Group 3-Diabetic+*P. americana* (250 mg/kg)

Group 4-Diabetic+*P. americana* (500 mg/kg)

Group 5-Diabetic+Metformin (500 mg/kg)

Twenty rats were fasted overnight, baseline blood glucose levels determined, before the animals were induced with diabetes by a single intraperitoneal (IP) injection of freshly prepared 150 mg/kg of alloxan monohydrate solution (Sigma St. Louis, M.O., USA) (Yanarday and Colac, 1998). Animals were considered diabetic if the blood glucose values were $\geq$ 200 mg/dl 48 hours after alloxan injection. Blood glucose levels were checked using a glucometer (Bioland glucometer, Germany).

Diabetic rats of Groups 3 and 4 were treated with low and high doses (250 mg/kg and 500 mg/kg) respectively of *P. americana* ethanolic extract orally twice daily, while Metformin group was administered once daily (500 mg/kg) for 21 days with the aid of a calibrated syringe with attached rubber cannula. Blood glucose levels checked every three days during the treatment period of 21 days by also picking the tail vein of the animals after which the animals were fasted overnight, rendered unconscious under pentobarbital anesthesia and sacrificed. Blood withdrawn via cardiac puncture was collected in heparin bottles 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. Serum very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) were calculated using Friedewald method.

Qualitative Phytochemical Analysis of *Persea americana* ethanolic Extract

Using standard methods, the extract was screened for bioactive ingredients. Saponins were determined by the method of Kokate KC 1997. Alkaloids were determined by method of Trease and Evans 1987, Tannins were determined by the method of Trease and Evans. Phytates were determined by method of Harbone 1973. Phenol was determined by the method of Trease and Evans 1987. Oxalates were determined by the method of Kokate 1997. Steroids were determined by the method of Harbone 1973. Cardiac glycosides were determined by the method of Harbone JB 1973.

Statistical Analysis

This was carried out using window SPSS (version 15.0). Data was analysed using one-way ANOVA and difference between groups compared using Least Significance Difference (LSD). Data was expressed as mean ± standard error of mean and values of P<0.05 were considered significant.

RESULTS AND DISCUSSION

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), the different doses of the *P. americana* extract and the standard drug (Metformin). Both doses of *P. americana* ethanolic 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 2 (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 group 2 (29.3 ± 4.0 mmol/l)

Table 1. Blood glucose levels of alloxan-induced diabetic animals treated with ethanolic extract of *P. americana* and Metformin. Values are expressed in mean ± SEM, n=5.*a=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 inducement with diabetes and commencement of treatment. Final=at 2-3 weeks after inducement with diabetes and treatment.

Groups	Blood glucose levels (mmol/l)	
	Initial	Final
Normal Control[1]	6.70 ± 0.8	7.5 ± 0.6
Diabetic Control[2]	16.65 ± 5.0*a	29.3 ± 4.0*a
Diabetic+*P. americana* (250 mg/kg)[3]	20.10 ± 7.0*a	14.4 ± 2.0bc
Diabetic+*P. americana* (500 mg/kg)[4]	20.40 ± 5.0*a	9.4 ± 1.0b
Diabetic+Metformin (500 mg/kg)[5]	25.20 ± 1.0*a	6.7 ± 0.0b

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 (11.3+1.1 mmol/l), Triglycerides (6.8+0.4 mmol/l) and low density lipoproteins (12.1+0.4 mmol/l) of the diabetic group 2 when compared to the normal control group 1 (TC=4.2 +0.1 mmol/l, TG=1.4+1.1 mmol/l, LDL=0.70+0.1 mmol/l) and the Metformin group 4 (TC=1.8+0.0 mmol/l, TG=0.7+0.3 mmol/l, LDL=0.63+0.1 mmol/l) (Table 2).

P. americana (500 mg/kg) diabetic treatment group 4 showed significant (P>0.05) decreases in TC (2.5+0.0 mmol/l), TG (1.9+0.1 mmol/l) and LDL (1.93+0.4 mmol/l), when compared with the diabetic control 2, (TC=11.3+1.1 mmol/l, TG=6.8+0.1 mmol/l, LDL=12.1+0.1 mmol/l). HDL-C also showed marginal increase in *P. americana* (500 mg/kg) diabetic treatment group 4 (2.8+0.5 mmol/l) (Table 2).

Table 2. Serum lipid profiles of diabetic rats treated with ethanolic extract of *P. americana* and Metformin. Values are expressed in mean ± SEM, n=5.*a=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.

Groups	Total cholesterol (mmol/L)	TG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)

Normal control1	4.2+0.1	1.47+1.1	1.9+1.5	0.70+0.1
Diabetic Control2	11.3+ 1.1*ac	6.8+0.4*ac	1.4+0.1	12.1+0.4*ac
Diabetic+*P. americana* (250 mg/kg)3	6.4+0.0bc	3.9+0.4bc	2.1+0.1	8.04+0.1bc
Diabetic+*P. americana* (500 mg/kg)4	2.5+0.0b	1.9+0.1b	2.8+0.5	1.93+0.4b
Diabetic+Metformin (500 mg/kg)5	1.8+0.0b	0.7+0.3b	3.1+0.2*ab	0.63+0.1b

Phytochemical screening of ethanolic extract of *P. americana*

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

Table 3. Phytochemical constituent of ethanolic extract of ethanolic extract of *P. americana*. Key: +=present; + +=moderately present; +++=highly present; -=absent.

Constituent	Inference	Quantitative Contents (%)
Alkaloids	++	1.4
Saponins	+	0.8
Tannin	++	1.3
Phytate	++	1.5
Phenol	+++	2.3
Cardiac-glycosides	+	0.5
Oxalate	+++	2.7
Steroids	+++	2

DISCUSSION

Uncontrolled Diabetes can lead to serious micro and macro vascular problems on the long term Mohana L, 2012 in addition causing many chronic complications including blindness, heart disease and renal failure Mamun-or-Rashid A et al. 2014. A significant change occurs in the structure and metabolism of lipid in diabetes leading to lipid peroxidation associated with hyperlipidemia (Kooti et al., 2016). These findings were in line with our study as there were increases in blood glucose levels and some lipid profiles of the untreated diabetic animals.

The study showed that daily oral administration of different doses of ethanolic extract of *Persea 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 *Persea americana* exerts significant hypoglycemic effects on alloxan induced diabetic rats. The research of (Kooti et al., 2016) also showed the abilities of some plants extract in reducing blood glucose levels in diabetic rats, which is in line with this research work. Hossain et al. 2010 reported antidiabetic and glycogenesis effect of plant fractions of Magnifera indica leaf extracts that produced significant restoration of blood glucose levels, improved oral glucose tolerance and increased liver glycogen synthesis activity in alloxan induced diabetic rats which is also in consonance with this research work. The significant anti-diabetic activity of the ethanolic extract of *Persea americana* may be 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 b3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor and peroxisome proliferators Broadhurst CL (1997).

Results of this study also showed the ability of the *Persea americana* ethanolic extract to significantly reduce TC, TG and LDL in contrast with increased HDL levels in diabetic treated animals. Research has shown that 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 outcome was in line with findings by Olagunju et al. 2017 who reported that cardiovascular disease marker on TC/HDL ratio of male albino albino rats fed with aqueous and ethanolic extracts of *Persea americana* fell within the low risk acceptable range and boosting the “good cholesterol” (HDL) which is good for cardiovascular health. Recent research also showed the abilities of chloroform and ethanolic fractions of Nauclea latifolia significantly reducing TC, LDL and VLDL in the treated diabetic albino rats after 2 weeks (Effiong and Essien, 2014). Therefore, the significant hypolipidemia and hypocholesterolemia activity of *Persea americana* ethanolic extract in this study can be attributed to the presence of phytochemicals such as alkaloids, phenols, saponins and sterols in the extract (Bopana KN 1997 and Katsumata KY 1999).

Results obtained from qualitative phytochemical screening of the *Persea americana* ethanolic extract shows it contains saponins, alkaloids, Steroid, phytate, phenol, oxalate, tannins and Glycosides, most of which are highly present, suggesting to contribute to the

therapeutic efficacy/anti-diabetic properties of the plant extract.

In conclusion, the administration of *Persea americana* ethanolic extract produced significant reduction of diabetes complications by restoration of blood glucose levels and eliminating lipid related complications associated with type I diabetes mellitus in alloxan-induced diabetic rats. Thus in the light of these study, further pharmacological investigations are needed to determine the chemical compositions of the extract and their exact mechanism of actions in the management of diabetes.

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