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Effect of Ethanolic Leaf Extract of *Psidium guajava* Linn. (Guava) in Alloxan-Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author RINU designed the study, wrote the first draft of the manuscript and wrote the protocol. Author PCO managed the literature searches, managed the analyses of the study and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Effect of water – soluble components of ethanolic leaf extract of *Psidium guajava Linn*. on blood glucose and cholesterol levels in alloxan-induced diabetic male albino wistar rats was evaluated. A total of 30 male albino wistar rats were randomized into 4 groups. Ten (10) animals each were randomly assigned to groups 1 and 2 and 5 each for groups 3 and 4. Group 1 animals were induced with 150 mg of alloxan/kg body weight of animal, after which they were administered 400 mg of extracts/kg body weight of animal. Group 2 animals serving as diabetic control were administered only 150 mg of alloxan/kg body weight of animal, no extract was given. Group 3 animals received only 400 mg of extract/kg body weight of animal while Group 4 animals served as normal control and were given distilled water in place of the extract. Extracts were administered twice daily and treatments lasted for 10 consecutive days. Blood samples were collected for analysis every two days from the tail tips of the rats. Results obtained showed that there were significant ($p < 0.05$) reductions in blood glucose and cholesterol levels in the diabetic treated rats (Group 1) when compared with the diabetic control rats (Group 2). However, administration of the extract did not show any hypoglycaemic effect on the normal rats ($p > 0.05$) compared with the control. The results suggest that leaf extract of *P. guajava Linn*. is antihyperglycaemic and not hypoglycaemic. In view of the fact that it also showed significant antihypercholesterolaemia, *P. guajava Linn*. may be a potential antidiabetic agent.

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1. INTRODUCTION

Diabetes mellitus is a metabolic disorder that is characterized by hyperglycemia and alterations in carbohydrate, fat and protein metabolisms associated with absolute or relative deficiencies in insulin secretion and/ or insulin action [1]. The causes are not completely understood, but a more sedentary lifestyle, the consumption of an energy rich diet, obesity, a longer life span [2], long-term diabetes, poor glycemic control, hypertension and dyslipidemia are linked to diabetes-related vascular complications [3].

Elevated levels of both fasting and post-prandial blood sugar is the specific characteristic of diabetes. Auto-immune and non-auto-immune responses cause destruction of pancreatic β-cells resulting in type 1 diabetes [4]. There are circulating immune markers against pancreatic islets known as anti-islet cell antibodies or β-cell antigens [5]. That is the reason for patient's dependency on external supply of insulin but those suffering from Type II diabetes known as non-insulin dependent, cannot properly respond to insulin. Type II diabetics can be treated by changes in diet intake, exercise and by use of medicines. Type II diabetes is the more prevalent form among the two and constitutes about 90% of the whole. Symptoms can be same for both types such as: (i) raised levels of blood glucose; (ii) increased need to drink water; (iii) repeated urine output; (iv) increased food intake and weight loss; (v) blurred vision; (vi) nausea and vomiting; (vii) fatigue and weakness; (viii) restlessness and changes of mood etc.

Diabetes is characterized by a high incidence of cardiovascular disease [6]. The primary goal of diabetes treatment is the prevention of macrovascular complications (myocardial infarction, heart failure, ischemic stroke), as well as the microvascular complications (retinopathy, neuropathy, and nephropathy); for that reason, most patients require not only a good glycemic control but also treatment for dyslipidemia [7].

Alloxan is administered parenterally: Intravenously, intraperitoneally or subcutaneously, while the dose necessary to induce diabetes is a function of the animal species, nutritional status and route of administration. This diabetogenic agent causes an insulin-dependent diabetes mellitus with characteristics similar to type I diabetes in humans [8,9]. It has been employed in experimental models for the study of diabetes.

Plants are sources of potential therapeutic agents against various diseases due to their biodiversity and the presence of a wide array of bioactive phytochemicals and secondary metabolites [10]. Several investigations into the chemical and biological activities of plants have yielded compounds with properties useful for the development of modern synthetic drugs for management of several diseases including diabetes [11]. In fact, there has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy accessibility and lesser side effects [12]. *Psidium guajava Linn.* (Family: *Myrtaceae*) is an economically important plant of high medicinal value [13,14,15]. *Psidium guajava* is commonly known as guava. It is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, anti-ulcer, analgesic, anticough, antimicrobial, antiplasmodial, antioxidant, anti-diabetes, anti-hypertensive, and for reducing fever [16]. In this study, the effect of the water – soluble components of ethanolic leaf extract of *P. guajava* on blood glucose and cholesterol levels in alloxan-induced diabetes in male albino wistar rats was evaluated.

2. MATERIALS AND METHODS

2.1 Plant Material

Fresh leaves of *Psidium guajava Linn.* were harvested from the premises of University of Benin, Benin City, Nigeria, during the month of June. The leaves were authenticated at the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria.

2.2 Extract Preparation

Ethanolic extract of the plant was prepared by adding 5 litres of absolute ethanol (BDH, England) to 500 grams of plant powder with repeated stirring for 3 days. The extract was then filtered and the recovered filtrate was concentrated at 60℃ using a rotary evaporator (Buchi Labortechnik, Flawil, Switzerland). The semi-solid extract was further dried to a constant weight by evaporation in a water bath at 60℃ and the yield determined. 5 grams of the extract was dissolved in 100 ml of distilled water to form the extract stock solution from which estimated

doses were administered. The doses of the extract administered was estimated using the method described by Tedong et al., [17].

2.3 Animals

Male albino wistar rats weighing 100–150 g were used. They were housed and maintained at room temperature with day/night cycles of 12 h in the animal house at the Department of Biochemistry, University of Benin. They were fed with standard rodent diet and water *ad libitum*.

2.4 Diabetes Induction

Diabetes was induced by intraperitoneal administration of freshly prepared alloxan (Aldrich, Germany) in a single dose of 150 mg/kg of body weight. Control rats were given distilled water only. Two days after alloxan administration, blood glucose levels were determined using a glucometer (Accu-Check, Mannheim, Germany) after an overnight fast (14 h) to confirm diabetes. Rats exhibiting blood glucose levels ≥ 200 mg/dl were considered for the study.

2.5 Experimental Protocol

Thirty (30) male albino wistar rats were used in this study. The rats were randomly divided into 4 groups of n animals each (n = number of animals).

Group 1 (n = 10): Diabetic rats treated with *Psidium guajava* extract (400mg/kg body weight) twice daily by gavage.

Group 2 (n = 10): Diabetic control rats

Group 3 (n = 5): Normal rats treated with *Psidium guajava* extract (400 mg/kg body weight) twice daily by gavage.

Group 4 (n = 5): Normal control rats.

The rats were acclimatized for a period of two (2) weeks, after which, they were fasted overnight (14 h) and blood collected from the tail tips of each animal for the determination of baseline values for glucose and cholesterol (pre-alloxan), followed by the induction of diabetes into some of the animals. After the 48 h adaptation period following alloxan administration, animals were fasted overnight (14 h) and blood samples collected (post-alloxan) and thereafter the extract was given for 10 consecutive days by oral administration using a gavage. Blood samples were collected (post-treatment) at intervals of 2 days.

For cholesterol determination, whole blood was collected into clean plain bottles and immediately placed on ice. The sample was allowed to stand for about 15 min and centrifuged (B. Bran Scientific, 80 – 2, England) at 10,000 r.p.m. for 5 min. Serum was separated from the clot with Pasteur pipette into sterile sample tubes for biochemical analysis.

2.6 Biochemical Analysis

The blood glucose concentration was estimated using One Touch Glucometer (Accu–Check, Mannheim, Germany) while cholesterol was determined spectrophotometrically after enzymic hydrolysis and oxidation as described by Randox reagent kit (Randox Laboratory Ltd, UK).

2.7 Statistical Analysis

The results were expressed as the mean ± SEM. All data were analyzed by the analysis of variance (ANOVA) followed by Duncan's multiple test using SPSS Advanced Statistical Version 21 package with statistical significance level at $p \leq 0.05$.

3. RESULTS

Table 1 shows the mean values of blood glucose of controls, normal and diabetic treated rats. The results revealed a significant ($p < 0.05$) increase in fasting blood glucose in the diabetic control group when compared with the normal control rats. A significant ($p < 0.05$) decrease was observed in the diabetic rats treated with the ethanolic leaf extract of *P. guajava*, however no such decreases were shown in the normal treated rats ($p > 0.05$).

Table 2 revealed the mean values of serum cholesterol of the control, treated and diabetic rats. The mean values of cholesterol were significantly ($p < 0.05$) higher in diabetic control rats when compared with the normal control rats. The cholesterol levels in the diabetic rats treated with the ethanolic leaf extract of *P. guajava* decreased significantly ($p < 0.05$) when compared with the control. There was no significant difference in serum cholesterol levels between normal treated rats with *P. guajava* extract and normal control rats ($p > 0.05$).

Table 1. Effect of water – soluble components of ethanolic leaf extract of *P. guajava* on blood glucose concentration in diabetic and non-diabetic male rats

Fasting blood glucose concentration (mg/dl)

Group	Pre - Alloxan	Post-Alloxan	Post - treatment								
			Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
1 n = 10	74.30 ± 7.66^{a}	460.90 ± 19.82^{c}	415.59 ± 1.30^{b}	397.13 ± 7.80^{b}	377.16 ± 7.30^{b}	345.15 ± 9.50^{b}	323.11 ± 1.40^{b}	297.13 ± 6.10^{b}	273.13 ± 0.00^{b}	263.20 ± 3.40^{b}	169.12 ± 7.60^{b}
2 n = 10	81.20 ± 9.11^{a}	401.16 ± 3.60^{c}	424.73 ± 7.70^{c}	388.14 ± 6.80^{b}	409.14 ± 4.50^{c}	395.12 ± 5.50^{c}	394.16 ± 3.00^{c}	424.20 ± 2.20^{c}	430.36 ± 3.00^{c}	422.30 ± 4.00^{c}	417.25 ± 6.60^{c}
3 n = 5	83.40 ± 5.54^{a}	96.10 ± 8.18^{a}	91.40 ± 2.49^{a}	83.30 ± 7.87^{a}	67.30 ± 9.72^{a}	84.30 ± 5.77^{a}	79.30 ± 5.70^{a}	75.30 ± 5.77^{a}	75.20 ± 5.37^{a}	65.20 ± 4.37^{a}	75.50 ± 3.60^{a}
4 n = 5	76.00 ± 11.42^{a}	82.33 ± 7.42^{a}	93.40 ± 2.49^{a}	81.40 ± 0.71^{a}	75.30 ± 6.69^{a}	73.20 ± 3.85^{a}	71.20 ± 3.30^{a}	86.20 ± 3.00^{a}	86.30 ± 0.00^{a}	76.30 ± 7.80^{a}	66.80 ± 7.90^{a}

Values are expressed as mean ± SEM; n = number of rats. Values with different superscripts in the same column are significantly different (p ≤ 0.05)

Table 2. Effect of water – soluble components of ethanolic leaf extract of *P. guajava* on serum cholesterol concentration in diabetic and non-diabetic male rats

Serum cholesterol concentration (mg/dl)

Group	Pre - Alloxan	Post-Alloxan	Post - treatment								
			Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13	Day 15	Day 17
1 n = 10	132.40 ± 7.66^{a}	224.70 ± 8.30^{b}	216.70 ± 3.00^{b}	211.50 ± 0.80^{b}	208.10 ± 1.90^{b}	199.70± 7.17^{b}	190.40 ± 5.70^{b}	178.70 ± 8.00^{b}	164.80 ± 6.21^{b}	147.50± 5.70^{a}	133.00 ± 1.54^{a}
2 n = 10	121.50 ± 9.11^{a}	260.50 ± 5.01^{c}	262.80 ± 4.40^{c}	254.14 ± 4.68^{c}	250.10 ± 4.45^{c}	265.10 ± 5.55^{c}	290.10 ± 6.20^{c}	224.20 ± 6.20^{c}	250.30 ± 6.30^{c}	264.30 ± 0.04^{c}	262.30 ± 0.12^{c}
3 n = 5	148.80 ± 5.54^{a}	140.10 ± 6.14^{a}	144.10 ± 4.00^{a}	139.30 ± 7.87^{a}	151.30 ± 9.72^{a}	147.30 ± 5.77^{a}	140.80 ± 5.22^{a}	156.30 ± 5.77^{a}	147.00 ± 6.34^{a}	149.20 ± 2.56^{a}	146.90 ± 1.97^{a}
4 n = 5	142.20± 14.50^{a}	146.80 ± 0.58^{a}	146.60 ± 7.11^{a}	147.90 ± 7.30^{a}	140.30 ± 5.63^{a}	142.80 ± 5.85^{a}	131.30 ± 3.55^{a}	145.30 ± 0.00^{a}	139.20 ± 3.50^{a}	140.30± 7.80^{a}	137.40 ± 1.75^{a}

Values are expressed as mean ± SEM; n = number of rats. Values with different superscripts in the same column are significantly different (p ≤ 0.05)

4. DISCUSSION

Scientific studies have revealed the potentials of plants as sources of novel therapeutic agents. The results of this study clearly indicated that the administration of ethanolic leaf extract of *P. guajava* produced antihyperglycaemic and antihyperlipidemic effects against alloxan–induced diabetes in rats. As shown in Table 1, the administration of *Psidium guajava* significantly reduced the blood glucose levels in diabetic rats when compared with the untreated diabetic rats. This suggests that the plant has antihyperglycaemic activities. The blood glucose was significantly increased in the diabetic control rats but the intervention with the extract tended to ameliorate the effect. A number of agents apart from insulin have been implicated in the control of hyperglycaemia, such as alkaloids and tannins [18]. During diabetes, the levels of serum lipids (cholesterol, free fatty acids and phospholipids) are usually elevated. The results in Table 2 indicate significant increases in cholesterol levels in the diabetic rats. These accumulate in blood to constitute hypercholesterolaemia of diabetes as evident in this study. The study showed that oral treatment with 400 mg/kg body weight of the ethanolic leaf extract of *P. guajava* exerted a progressive reduction on elevated levels of blood glucose and cholesterol. The presence of phytochemicals in the extract with reported antidiabetic activities may be responsible for the observed reductions in blood glucose and cholesterol levels in the diabetic rats treated *with P. guajava* [10].

5. CONCLUSION

Based on our results, administration of the water – soluble components of ethanolic leaf extract of *P. guajava* lowered the blood sugar and cholesterol levels in the alloxan-induced diabetic rats. The ethanolic leaf extract showed significant ($p < 0.05$) antihyperglycaemic activity in alloxan–induced hyperglycaemia without causing hypoglycaemia, showing that an over dose of the extract may not cause hypoglycaemia.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical guidelines and approval of the University of Benin, Benin - City, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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