In Vivo Antidiabetic Activity of Aqueous Extract of Psidium Quajava in Alloxanised Diabetic Mice

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INTRODUCTION

Diabetes is a metabolic disorder of the endocrine system characterized by excessive plasma glucose levels emanating from hormonal imbalances or loss of cellular responses to hormones. Diabetes remains a pathological concern in public health today since associated with prolonged term destruction, malfunction, and impairment vital organs, moreso the kidneys, nerves, eyes, heart, and the vascular system. Studies have confirmed a steady rise in the prevalence of diabetes mellitus globally. The increase has been escalated by the aging population in the developed countries, a rise in cases of obesity as well as stressful life style [1]. The global increase is estimated to be at 6% annually. The World Health Organization (WHO) indicated that nearly 150 million people worldwide had diabetes mellitus by 1995, and the figure is projected to double by 2025 [2]. International Diabetes Federation (IDF) estimates showed that by 2007, approximately 246 million people had diabetes and is suspected to rise to 380 million by the year 2025 [3]. Statistics by the World Health Organization (WHO) infer that 347 million people have been tested for diabetes with 80% of them living in low and middle income countries [4]. The clinical manifestations of diabetes include; excessive thirst (polydipsia), frequent urination (polyuria), constant hunger (polyphagia), weight loss, blurred vision and fatigue. These symptoms are more profound in type I diabetes than in type II diabetes. Consequently, type II DM, may be diagnosed several years after occurrence [5]. Uncontrolled diabetes is associated with increased susceptibility to infections such as skin sepsis (boils) or genital candidiasis, and complains of pruritus vulvae or balanitis [6]. Diabetics can, over time, experience nerve damage, though with some, the damage may be non-symptomatic. Estimates by WHO shows that approximately 5% of the world prevalence of blindness is due to diabetic retinopathy, with projections of 15%-17% in developed countries [7]. Several blood test protocols are routinely employed in diagnosis of diabetes mellitus. Repeated fasting plasma glucose greater than 126 mg/dL is strongly suggestive of diabetes, with values from 100 to 126 mg/dL been suggestive of impaired fasting glucose [8]. Two postprandial tests with glucose levels of 200 mg/dL or higher after two hours are suggestive of diabetes. Glycosylated hemoglobin between 5.7% and 6.4% is indicative of elevated chances of diabetes. Levels ranging from 6.5% and above denote diabetic state. The orthodox antidiabetic agents are not only unaffordable and unavailable but may still exhibit adverse side effects which are associated with diabetes complications. Majority of the population in developing countries have therefore embarked on traditional and herbal medicines since they are locally available, cheap, and purported to be safe. Estimates by the World Health Organization indicate that about 80% of the world populations depends on traditional medicine for their primary health care. [9]. Aqueous extract of *Psidium guajava* has been reported as a folkloric herb in management of diabetes mellitus even though the claim has not been validated scientifically. Moreover, there is considerable evidence indicating that many of the plant...
products are toxic to humans, [10] hence every herbal product requires thorough testing. Since many conventional drugs trace their origin from medicinal herbs, research on these plants may help in discovery of new drugs that are more efficacious and safer. Moreover, human activities are rendering many plants extinct, which make it crucial to study them, and advocate for their conservation. Therefore, this study was undertaken to determine in vivo antidiabetic activity of aqueous plant extract of _Psidium quajava_ in alloxanised diabetic male Swiss albino mice.

**Materials and methods**

**Study site**

This study was executed at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University, Kenya.

**Collection and processing of plants materials**

Stem bark and leaves of _Psidium quajava_ were obtained from the plant’s natural habitat from Teret location, Njoro Division in Nakuru County, Kenya. The local identification of the plant, the locality where it grows, the part of the plant to be collected, time when bioactivity is at peak and the method of preparation was provided by a Herbalist. The plant was authenticated by a taxonomist at the East African Herbarium, National Museum of Kenya.

**Preparation of the plant sample**

The plants samples were cut into small pieces, dried at room temperature until completely dry. The dried plants materials were ground by a mechanically into a fine powder and sieved through a 40mm mesh sieve. The obtained powders was kept in air tight plastic bags. One hundred grams of each powdered plant sample was extracted in one litre of distilled water at 60°C till time for bioassay. The extract was decanted into a clean conical flask and sieved through a Whatman filter paper into another conical flask. The filtrate was then freeze dried and stored in a freezer at -20°C till time for bioassay.

**Experimental animals**

This study employed male Swiss albino mice (3-5 weeks old) weighing 19-24g. The animals were bred at the Department of Biochemistry and Biotechnology of Kenyatta University. The experimental mice were housed at room temperature with 12hours/12hours darkness photoperiod, and provided with rodent standard pellets and water _ad libitum_. The study was certified by the ethics committee for the care and use of Laboratory Animals of Kenyatta University, Kenya.

**Experimental design**

Antidiabetic activity of the aqueous plant extract was investigated in alloxanized diabetic mice. The mice were divided randomly into two categories. The first category was employed for hypoglycemic study through oral administration of the plant extract. It composed of the following groups of five mice each; Group I composed of normal mice (reference) administered with 0.1ml of normal saline; Group II comprised of diabetic mice dosed with 0.1ml of normal saline; Group III composed of diabetic mice (positive control) administered with Glibenclamide (reference drug at 3mg/kg body weight) in 0.1 ml normal saline; Group IV composed of diabetic experimental mice dosed with plant extract at 50mg/kg body weight in 0.1 ml normal saline; Group V composed of diabetic experimental mice administered with plant extract at 100mg/kg body weight in 0.1 ml normal saline; Group VI composed of diabetic experimental mice administered with plant extract at 200mg/kg body weight in 0.1 ml normal saline and Group VII composed of diabetic experimental mice, administered with plant extract at 300mg/kg body weight in 0.1 ml normal saline.

The second category was used for antidiabetic assay through intraperitoneal administration of the plant aqueous extract. A replica of experimental design as that employed with the first group was used except that Insulin was used as a reference intraperitoneal drug (at 1 IU/kg body weight).

**Induction of hyperglycemia**

Hyperglycemia was induced intraperitoneally through administration of freshly prepared 10% alloxan monohydrate at 186.9mg/kg body weight. Forty eight hours after administration of alloxan monohydrate, blood glucose levels were profiled using a glucometer. Mice with glucose levels above 11.1mmol/litre were considered diabetic and appropriate for use in the bioassay. The animals were fasted for 8-12 hours prior to execution of the experiment [11], though were allowed free access to water.

**Blood glucose assay**

Blood used for glucose assay was obtained by sterilizing the tail (with 10% alcohol) before nipping the tail at the start of the experiment and repeating the same after 1, 2, 3, 4, 6 and 24hrs. The glucometer model (HYPOGAURD, ENGLAND), was employed in determining blood glucose levels.

**Phytochemical screening**

Phytochemical assay of _Psidium quajava_ was performed using standard procedures to quantitatively establish the levels of saponins, tannins, flavanoids, alkaloids and total phenols.

**Data analysis**

The data obtained was escorted to the Microsoft Excel Spread Sheet where it was cleaned and then transferred to Statistical Package for Social Sciences Software (SPSS) for statistical analysis. The results of statistical analysis were expressed as Mean ± Standard Deviation (SD). One-way ANOVA and post-ANOVA (Tukey’s post hoc test) were used to compare the means of untreated group of normal mice with diabetic groups of mice treated with normal saline, conventional drug and plant extract at various dosages. Statistical significance was set at p ≤ 0.05.

**RESULTS**

**4.1.3 Effects of orally and intraperitoneally administered of aqueous leaf extract of _P. quajava_ at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels**

The stem bark extract of _P. quajava_ yielded 5.30% (w/w) brown powder. Intraperitoneal administration of aqueous extract of _P. quajava_ at four therapeutic doses in alloxan induced diabetic mice lowered blood glucose levels from the 1st hour to the 4th hour in a dose dependent manner (Table 4.5; Figure 4.5). This occurred in three phases; in the first hour, the extract caused a steep decline in blood glucose levels, followed by a steady decline from the second to fourth hour. A steady increase was then observed from the 6th hour through to the twenty-fourth hour. In the 1st hour the four therapeutic doses of the extract lowered blood glucose levels by 28%,
33%, 26%, and 42% respectively, compared to insulin which lowered blood glucose levels by 38% within the same hour. By the 4th hour the extract had lowered the blood glucose levels by 65%, 53%, 64% and 73% in all the four therapeutic doses respectively, compared to insulin, which had lowered blood glucose levels by 74% by the same hour. By the 6th hour, however, only the dose of 300 mg/kg body weight had sustained blood glucose level within normal and was as effective as insulin.

Oral administration of aqueous extract of *P. quajava* at four therapeutic doses in alloxan induced diabetic mice decreased the blood glucose levels in a dose independent manner. During the 1st hour, the reduction in blood glucose levels by the four therapeutic doses were 34%, 34%, 34%, and 38%, respectively, compared to glibenclamide (reference drug) which lowered blood glucose level by 36% within the same hour (Table 4.6; Figure 4.6). By this hour, the four tested doses did not lower blood glucose levels to normal. By the 4th hour, the four therapeutic doses of the plant extract lowered blood glucose level by 48%, 50%, 60%, and 54%, respectively, compared to glibenclamide which had lowered blood glucose level by 60% by the same hour. By this hour the four doses did not lower blood glucose levels to normal. However, by the 6th hour, the doses of 200 mg/kg body weight and 300 mg/kg body weight had lowered blood glucose levels to normal and were as effective as glibenclamide (reference drug). Thereafter, a steady increase in blood glucose levels was observed through to the twenty fourth hour, in all the test doses.

Table 1: Effects of intraperitoneal administration of aqueous plant extract of *P. quajava* at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose Levels at Varying Times (mM)</th>
<th>0hr</th>
<th>1hr</th>
<th>2hr</th>
<th>3hr</th>
<th>4hr</th>
<th>6hr</th>
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<tr>
<td>Normal/Saline</td>
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<td>5.42±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.24±0.15&lt;sup&gt;ab&lt;/sup&gt;</td>
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<td>19.52±2.5&lt;sup&gt;abc&lt;/sup&gt;</td>
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Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at p<0.05 by ANOVA and post ANOVA. Means along each row followed by similar upper case letters are not significantly different at p<0.05 by ANOVA and post ANOVA.

Table 2: Effects of oral administration of aqueous plant extract of *P. quajava* at four therapeutic doses in alloxan induced diabetic mice on blood glucose levels

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<th>Treatment</th>
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Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at p<0.05 by ANOVA and post ANOVA. Means along each row followed by similar upper case letters are not significantly different at p<0.05 by ANOVA and post ANOVA.
Figure 1: Mean percentage change in blood glucose levels by aqueous extracts of *P. quajava* at four therapeutic doses intraperitoneally administered in alloxan induced diabetic mice

Figure 2: Mean percentage change in blood glucose levels by aqueous extracts of *P. quajava* at four therapeutic doses orally administered in alloxan induced diabetic mice

Quantitative estimation of phytochemicals present in *P. quajava* aqueous plants extracts

The quantitative estimation of phytochemicals in the plants extract under study is shown in Table 3.

Results indicate that the plant extract of *P. quajava* contained flavonoids, total phenols, alkaloids, tannins and saponins at varying concentrations.

Table 3: Phytochemical composition of *P. quajava* plants extracts

<table>
<thead>
<tr>
<th>Phytochemical Content (mg/g)</th>
<th>Tannins</th>
<th>Total Phenols</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Alkaloids</th>
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</table>

Results are expressed as Mean± standard deviation (SD).
Discussion

The alloxanised diabetic mice exhibited a two- to five-fold elevation of blood glucose (192.10mg/dL to 498.2mg/dL) compared to the normal control mice. Alloxan monohydrate induces a selective destruction of the pancreatic β-cells through the formation of free radicals such as like nitric oxide [12]. The aqueous plant extract of *P. quajava* demonstrated blood glucose lowering ability when orally and intraperitonially administered. The blood glucose lowering effect of this plant was in tandem with reported literature of other plants already researched. Ethanol and ethylacetate extracts of *Swertia punicea* demonstrated hypoglycemic effects in streptozotocin-induced diabetic mice [13]. Administration of Ethanolic extract of *Pandanus odorat*us at 0.005g/kg elevated serum insulin levels and hepatic glycogen levels in healthy rats [14]. Water extract of *Eucalyptus globulus* administered in mice (at 500mg/L of solution), enhanced peripheral glucose utilization in abdominal muscle and elevated insulin secretion by the pancreatic beta cells [15]. Numerous mechanisms of action have been put across for these plant extracts. Some attribute their effects on increase in insulin sensitivity, increased inhibitory effect against insulinase, while other associate their activity on enhancement of the synthesis, release, regeneration or revitalization of the pancreatic β- cells. Improved glucose homeostasis has also been proposed as a possible mechanism of action. The hypoglycemic activity of the plant extracts could be due the presence of the alkaloids, saponins, polyphenols, tannins, flavonoids and steroids [16]. The phytochemical assay of *P. quajava* confirmed the presence of flavonoids, alkaloids, saponins, total phenols and tannins.

Saponin containing *Kalopanax pictus* has been shown to exhibit hypoglycemic activity in streptozotocin-induced diabetic rats [17]. In other studies, saponins have been shown to lower blood glucose levels in elderly diabetic patients [18]. Quercetin (aflavonoids) when intraperitonially administered in streptozotocin-induced diabetic mice, resulted in suppression of glucose levels, reduction of plasma cholesterol and triglycerides and significantly elevated hepatic glucokinase activity. Bassic acid, a triterpene isolated from *Bunelia sartorum* Mart was found to exhibit significant antidiabetic activity in alloxanized diabetic mice [19]. Jamboline, a glycoside extracted from *Syzygium cumini* (seeds), demonstrated antidiabetic activity [20]. The blood glucose lowering effect of the aqueous extracts of *P. quajava* in a non-dose dependent manner may imply uptake of the active ingredients through saturable active transport, where a specific concentration saturation of the extract occurred resulting to the rest of extract being excreted or it may also reflect maximum hypoglycemic activity at the lowest dose used (50mg/kg body weight).

Conclusion

This study demonstrated that *P. quajava* posses hypoglycemic effects in alloxan-induced diabetic mice, thus scientifically validating its continued use in the management of diabetes mellitus. The antidiabetic activity was due to cumulative effect of phytochemicals present in the plant extract including alkaloids, tannins, flavonoids, saponins and total phenols. However, further research should be done focusing on isolating the bio-active molecules responsible for the hypoglycemic effect of this plant through bioassay guided fractionation. Moreover, the antidiabetic activity of the organic solvent extraction of this plant should also be assayed, to compare the activities of both aqueous and organic fractions.

Acknowledgements

The author wish to acknowledge the Department of Biochemistry and Biotechnology, Kenyatta University for permitting me to use the Departmental animal house facility for mice breeding and performing the efficacy studies; the technical support from Mr. James Adino of the Department of Medical Laboratory Sciences, Kenyatta University for assisting in handling the laboratory mice used in the research.

REFERENCES


