Hypoglycemic and Hypolipidemic Potentials of Isolated Fraction of *Psidium Guajava* Leaf In Alloxan-Induced Diabetic Rats

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**ABSTRACT**

Diabetes is a chronic metabolic disorder with impaired glucose tolerance and high risk of cardiovascular disease. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations. There is a constant attempt by scientists to understand the active principles present in *Psidium guajava* with antidiabetic properties. The present study examines the hypoglycemic and hypolipidemic effect of isolated fraction of *Psidium guajava* in alloxan-induced diabetic rats. Diabetes was induced by alloxan. Blood glucose and lipid profile levels were measured. A significant decrease in blood glucose, total cholesterol, triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, and a significant increase in high-density lipoprotein cholesterol, were observed after 21 days treatment of isolated fraction of *Psidium guajava* leaf. Further characterizations of this active component of *Psidium guajava* leaf for diabetes are warranted.

**Keywords**: *Psidium guajava*, diabetes, total cholesterol, triglyceride, HDL cholesterol

**INTRODUCTION**

Diabetes mellitus is a metabolic disease, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels, such as lipids and proteins \(^1\). This metabolic disorder is the result of a deficiency in insulin secretion or a resistance to insulin action, or both \(^2\). According to current estimates world-wide prevalence of diabetes mellitus in 2008 was reported to be more than of 347 million with varying prevalence among different ethnic groups \(^3,4\) and this number is expected to reach 500 million by 2025. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to

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provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural products from plants. Furthermore, some drugs have various and severe adverse effects. Therefore, products of natural origin with no or very few side effects are desirable as substitutes for chemical therapeutics.

Bio-flavonoids comprise a group of phenolic secondary plant metabolites that are widespread in nature. Bio-flavonoids are well-known for their multi-directional biological activities including antidiabetic efficacy. Psidium guajava Linn, belonging to the family of Myrtaceae, has been used as health tea. There is a constant attempt by scientists to understand the active principles present in Psidium guajava with antidiabetic properties. The present study examines the hypoglycemic and hypolipidemic effect of isolated fraction of Psidium guajava in alloxan-induced diabetic rats.

MATERIALS AND METHODS
Collection and preparation of plant material
Fresh leaves of Psidium guajava were collected in Coimbatore, during the months of April-May. The plant was authenticated by Botanist at the Government Arts College, Coimbatore. Plant material was dried under shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes, then stored in airtight closed bottles until required.

Extraction and isolation
The coarse powder (100 g) was extracted successively with ethanol (250 ml) by hot continuous percolation method in a Soxhlet apparatus for 24 hrs. The extracts was concentrated and re-concentrated in petroleum ether (40°-60°C) (fraction-I), diethyl ether (fraction-II) and ethyl acetate (fraction-III) in succession in the ratio of 1:2:1. Each of the steps was repeated three times to ensure complete extraction in each case. Fraction I was rejected since it was rich in fatty substances whereas fraction II was analysed for the free flavonoids in each of the samples. Fraction III of each of the test samples was hydrolysed by refluxing with 7% H2SO4 (10 ml/gm residue) for 5 hrs. The mixture was filtered and the filtrate extracted with ethyl acetate in a separating funnel. The ethyl acetate layer was washed with distilled water till neutrality and dried in vacuo. The residues were taken up in small volumes of ethanol separately and then the fraction was subjected to TLC to confirm the isolated fraction (quercetin).

Introduction of experimental diabetes
Hyperglycemia was induced by intraperitoneal injection of freshly prepared aqueous solution of alloxan monohydrate (SD fine Chemicals Pvt. Ltd., Biosar) 150 mg/kg, to overnight fasted rats except control group. Diabetes was confirmed after 48 hour, animals with plasma glucose level above 150 mg/dl (diabetic) were selected for the study. The diabetic animals were allowed free access to tap water and pellet diet and were maintained at room temperature in plastic cages.

Experimental design
Animals were classified into three groups of six rats each. Group I: served as control and received normal saline (2
ml/kg body weight). Group II: treated with alloxan monohydrate 150 mg/kg served as diabetic control. Group III treated alloxan monohydrate (150 mg/kg) and isolated fraction of *Psidium guajava* 10 mg/kg body weight). At the end of the treatment period (21st day), the animals in all the groups were sacrificed, dissected, and bled using the orbital technique and cardiac puncture. Blood samples for the different tests were collected.

**Sample preparation for biochemical estimations**

The blood sample was allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30°C for 15 min. Haemolysis-free serum samples were stored at -70°C before determination and utilized for the estimation of various biochemical parameters. Livers were excised, washed thoroughly in ice-cold saline to remove the blood. They were then gently blotted between the folds of a filter paper and weighed in an analytical balance. Ten percent of homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogenizer at 20°C. The homogenate was centrifuged at 3000 g for 20 min to remove the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for further hepatic biochemical assays.

The level of glucose in serum was analyzed by the method of Sasaki and Matsui. Total cholesterol (TC) by the method of Parekh and Jung, triglycerides (TG) by the method of Foster and Dunn and high-density lipoprotein (HDL) cholesterol by the method of Gordon. Low-density lipoprotein (LDL) cholesterol values have long been estimated using the Friedewald formula:

\[
[\text{TC}] - [\text{total HDL cholesterol}] - 20\% \text{ of the TG value} = \text{estimated LDL cholesterol.}
\]

The very low-density lipoprotein (VLDL) cholesterol is estimated as one-fifth of the TG.

**Statistical analysis**

Data represent the mean ± standard deviation (S.D.) of the indicated number of experiments. In the present investigation, since more than two treatment groups have been studies. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Duncan’s multiple range test (DMRT) by using statistical package of social science (SPSS) version 12.0 for windows. \(P\) values \(<0.05\) were considered as level of significance.

**RESULTS**

The hypoglycemic and hypolipidemic activity of isolated fraction of *Psidium guajava* leaf was shown in Table 1. The concentration of glucose was significantly higher \((p<0.001)\) in alloxan treated rats (Group II), as compared to normal control animals (Group I). Subsequently, these values were found to attain a near \((p<0.001)\) to control group in alloxan plus isolated fraction of *Psidium guajava* treated rats (Group III).

The concentration of TC, TG, LDL cholesterol and VLDL cholesterol were significantly \((p<0.001)\) higher in alloxan treated rats, as compared to normal control animals. These constituents were found to attain a near normal \((p<0.001)\) level in alloxan plus isolated fraction of *Psidium guajava* treated rats. The levels of HDL cholesterol was noted a significant decrease \((p<0.001)\) in alloxan administered
rats, when compared with normal controls. There was a significant (p<0.001) increase in HDL cholesterol in alloxan plus isolated fraction of Psidium guajava treated rats.

**DISCUSSION**

This study was designed to investigate the effects of isolated fraction of Psidium guajava ethanolic leaf in alloxan induced diabetic models. Increased plasma concentrations of glucose in alloxan treated rats observed in this study. The most common pattern of dyslipidemia was also noted in alloxan treated diabetic models.

Alloxan is the most prominent diabetogenic chemicals in diabetes research. It is a very unstable chemical compound with a molecular shape resembling glucose. Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane. The alloxan molecule is structurally so similar to glucose that the GLUT2 glucose transporter in the beta cell plasma membrane accepts this glucomimetic and transports it into the cytosol. 

In the current investigation, we examined fraction compound of plant extract to protect against alloxan-induced diabetes. Plant extract fraction compound (10 mg/kg) treated alloxan-induced diabetic rats’ significantly decreased glucose, TC, TG, LDL cholesterol and increased in HDL cholesterol and level was observed. The TLC pattern compared with standard quercetin clearly depicted the presence of quercetin in the ethanolic extract of the leaves of Psidium guajava. The results of our study revealed that the lower level of glucose and lipid profile in the plant was probably associated with high content in quercetin in Psidium guajava leaf extract and confirming thereby its usefulness for diabetic patients. The findings of our study are in agreement with the findings of others.

Glucose tolerance tests of the diabetic animals approached those of normal rats, their plasma glucose, TC and TG were reduced significantly, while their hepatic glucokinase activity was significantly increased upon quercetin treatment. It was also noted that the number of pancreatic islets significantly increased in both treated normal and diabetic groups. Kanter et al. results support that the quercetin treatment may decrease blood glucose and increase plasma insulin, calcium, and magnesium.

Li et al. results indicated that quercetin, isoquercetin and rutin could bind alpha-glucosidase to form a new complex and the sequence of binding constants was quercetin > isoquercetin > rutin. Cheng et al. suggest that quercetin in the aqueous extract of Psidium guajava leaves promotes glucose uptake in liver cells, and contributes to the alleviation of hypoglycemia in diabetes as a consequence. Oral administration of quercetin (30 mg/kg) to diabetic rats for a period of 30 days showed a decrease of plasma glucose and increase in insulin levels were observed along with the restoration of glycogen content and the activities of carbohydrate metabolic enzymes in quercetin-treated diabetic rats.

Nuraliev and Avezov, established that quercetin in doses of 10
and 50 mg/kg promotes normalization of the level of glycemia and blood coagulation, increases liver glycogen content, reduces high blood serum concentrations of TC and LDL cholesterol, seen in diabetes. The oxidation of LDL cholesterol can result in the formation of atherosclerotic plaques, leading to cardiovascular disease. However, several studies have illustrated quercetin’s ability to inhibit LDL cholesterol oxidation. Chopra et al. reported that the red wine extract and quercetin inhibited LDL cholesterol and there was no effect on plasma concentrations of vitamin C and E. Graf and co-workers found a 21% reduction in cardiovascular disease mortality when the intake of quercetin was greater than 4 mg/d. Torres-Piedra et al. results showed that flavonoids induced a significantly diminishing of TC, TG and LDL cholesterol and an augment of HDL cholesterol.

CONCLUSION
The present study suggested that *Psidium guajava* extract present major constituent’s of quercetin may be regulated blood glucose and lipid profiles in alloxan induced diabetic rats. Further characterizations of this active component of *Psidium guajava* leaf for diabetes are warranted.

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REFERENCES


Table 1 Effect of isolated fraction of Psidium guajava leaf extract on glucose and lipid profile in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Glucose (mg/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>High-density lipoprotein cholesterol (mg/dl)</th>
<th>Low-density lipoprotein cholesterol (mg/dl)</th>
<th>Very low-density lipoprotein cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>102.0±4.0\textsuperscript{a}</td>
<td>149.1±29.8\textsuperscript{a}</td>
<td>101.8±11.6\textsuperscript{b}</td>
<td>36.5±4.6\textsuperscript{b}</td>
<td>92.2±30.2\textsuperscript{a}</td>
<td>20.3±2.3\textsuperscript{b}</td>
</tr>
<tr>
<td>Group II</td>
<td>174.1±10.8\textsuperscript{b}</td>
<td>231.1±28.3\textsuperscript{b}</td>
<td>133.3±17.5\textsuperscript{c}</td>
<td>18.0±3.5\textsuperscript{a}</td>
<td>186.5±29.8\textsuperscript{c}</td>
<td>26.6±3.5\textsuperscript{c}</td>
</tr>
<tr>
<td>Group III</td>
<td>106.6±7.6\textsuperscript{a}</td>
<td>164.3±10.8\textsuperscript{a}</td>
<td>67.6±15.0\textsuperscript{a}</td>
<td>23.0±8.5\textsuperscript{a}</td>
<td>128.0±9.4\textsuperscript{b}</td>
<td>13.5±2.8\textsuperscript{a}</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common marking (a, b, c,) differ significantly at P<0.05 (DMRT)