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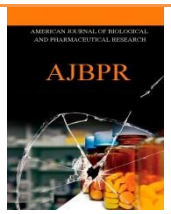

HYPOGLYCEMIC AND ANTIHYPERLIPIDEMIC POTENTIALITY OF *PSIDIUM GUAJAVA* LINN AGAINST STREPTOZOTOCIN (STZ) INDUCED DIABETICS IN RATS

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ABSTRACT

Diabetes mellitus is a metabolic disorder which results due to chronic hyperglycemia associated with the imbalance in carbohydrate, fat and protein metabolism. Presently available Several drugs reduce the hyperglycemia in diabetes mellitus; unfortunately these drugs have side effects. While, herbal drugs are mostly out of toxic or side effect than the chemical drug. Hence, The aim of the present study was designed to compare the possible therapeutic effects of *Psidium guajava* leaves extracts against streptozotocin (stz) induced diabetic rats. The ethanolic extract of *P. guajava* was administered orally in an aqueous solution at a dose of 500mg/kg body wt. to diabetic rats. Serum glucose level and lipid profile were estimated after administration of the extracts. Applied doses did not cause any acute toxicity or behavioral changes. The blood glucose levels was significantly ($P<0.001$) reduced when compared to the streptozotocin (stz) induced diabetic rats .The lipid profile such as Total Cholestol, Total Glycerides, Low Density Lipo proteins and Very Low Density Lipoproteins levels were significantly decreased in *P. guajava* treated diabetic animals. In contrast, High Density Lipoproteins levels were increased when compared to the Diabetic control rats. In conclusion, *P. guajava* leaves produced a significant hypoglycemic effect and also hypolipidemic activity at dose level of 500 mg/kg.

INTRODUCTION

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids, carbohydrates, proteins metabolism and it increases risk of cardiovascular diseases complications [1]. The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characteristics such as associations with obesity, age, and insulin. But, both types of the diabetes share the common characteristics of hyperglycemia, microvascular and macrovascular complications. Moreover, the alterations of lipoproteins metabolism are involved to the pathogenesis of the cardiovascular disease in both forms of diabetes in a similar way [2]. Diabetes has a considerable impact on the health, life style, life expectancy of patients and its related complications are major healthcare problems.

Currently, diabetes is controlled by handful of available drugs such as oral hypoglycemic agents and insulin, but they have their own limitations. Traditionally, many herbal medicines and medicinal plants have been

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used for the treatment of diabetes as an alternative medicine [3].

Presence of various phytoconstituents in medicinal plants is thought to act on a different series of targets by multiple modes and mechanisms. Hence, plants have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications [4]. Screening of medicinal plants is one of the alternative and valid approaches in the drug development process because they contain diverse phytoconstituents which may give new drug leads and may be effective and safe in diabetes. In India, traditionally numbers of plants are used to manage the diabetic conditions and their active principles were isolated but few plants have been scientifically studied. Therefore, the present study was carried out to evaluate the antidiabetic activity of *Psidium guajava* in STZ induced diabetes and to probe into the mechanism of its antidiabetic property.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately weighing 160-180g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The animal feed composition is crude protein (22.3%), crude oil (4.01%), crude fibre (4.02%), Ash (8.02%) and sand silical (1.02%). Ethical clearance was given by Institutional Animal Ethics Committee and conducted experimental rules of Indian National Science Academy (CPCSEA/265).

Chemicals:

Streptozotocin (STZ), Ethylene Diamine Tetra Acetic Acid (EDTA)), Glibenclamide (Prudence PharmaChem, India), Chloroform were purchased for Sigma chemical company, Mumbai All other chemicals and reagents used in this study was of analytical grade with high purity and were obtained from Glaxo laboratories and Sisco Research laboratories, Mumbai, India.

Preparation of plant extract

The leaves of *P. guajava* were collected from Sharmilla medicinal garden, Thanjavur (Fig.1). The collected leaves of *P. guajava* were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture. The powder material of *P. guajava* leaves were macerated with 70% ethanol at room temperature for 3 days. After 3 days, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45^{o}C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made up to a known volume in distilled water just before oral administration.

Streptozotocin (STZ) Induced Diabetic rats

The animals were divided into four groups of six animals each. Diabetes was induced in all groups except normal control following overnight fasting (deprived of food for16h allowed free access to water) by a single intraperitoneal injection of 65mg/kg of streptozotocin (STZ) dissolved in a freshly prepared 0.1M citrate buffer (pH4.5) (Liu et al., 2008).The animals of normal control (Group I) were injected with saline alone. Diabetes was confirmed 72 h after induction by measurement of tail vein blood glucose levels by glucose oxidase-peroxidase method using strips. Group II served as diabetogenic rats (Control). Group III rats treated with *P. guajava* at a dose of 500mg/kg was orally given once a day for 15 days after hyperglycemia was confirmed. Group IV rats treated with Glibenclamide as a standard at dose of 0.25mg/kg [5]. After complete the experimental period, the animals were killed cervical dislocation after an overnight fasting. The blood sample was collected. The blood was allowed to clot by standing at room temperature for 30 minutes and then refrigerated for another 30 minute. The resultant clear part was centrifuged at 3000 rpm for 10minutes and then the serum (supernatant) was isolated and stored at refrigerated until required for analysis.

Biochemical estimations

Serum glucose was estimated by the oxidase method [6]. The total cholesterol was estimated by Allain method [7]. Triglyceride was estimated by the Werner method [8]. HDL cholesterol was separated by adding phosphotungsti magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by Allain method. The concentration of LDL cholesterol was calculated by using the Friedwald formula [9] and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl). Heamoglobin estimated by the method of Dacie and Lewis [10].

Statistical Analysis:

Values were expressed as mean ± standard deviation for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for post-hoc multiple comparison tests. Statistical Package for Social Studies (SPSS) 9.0 version was used and $p<0.001$ was considered to be significant.

RESULTS

The ethanolic extract of *P. guajava* was administered orally in an aqueous solution at a dose of 500mg/kg body wt. to diabetic rats to assess the synergetic impact of the plant extracts. The plant extracts were fed with normal and diabetes induced rats. The blood glucose levels was significantly (P<0.001) reduced when compared to the specific diabetic control animals (Table 1).

The lipid profile such as TC, TG, LDL and VLDL levels were significantly increased in diabetic control animals (DC) where as HDL levels were decreased when compared to the control rats. The plant extracts were administered orally at a dose of 500mg/kg body wt., to diabetic rats significant (P<0.001) depletion in the total cholesterol, TG, LDL, and VLDL levels and increment of HDL levels were recorded in the diabetic animals (Table 1 and 2). The depleted high density lipoprotein (HDL) in the diabetic rats, increased significantly (P<0.001) after the administration of the plant extract. The plant extract possesses significant antidiabetic activity and close proximity to standard.

Table 1. Effect of *P. guajava* leaves extract on glucose, Hb, cholesterol and triglycerides in experimental rats

Treatment Groups	Glucose (mg/dl)	Hb (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Group I	101.69 ± 5.08	13.76 ± 0.68	90.96 ± 4.54	114.28 ± 5.71
Group II	206.32 ± 10.31[a]	9.03 ± 0.45[a]	236.36 ± 11.81[a]	188.57 ± 9.42[a]
Group III	96.61 ± 4.83[b]	15.49 ± 0.77[b]	76.36 ± 3.81[b]	82.85 ± 4.14[b]
Group IV (S)	98.30 ± 4.91[b]	13.35 ± 0.66[b]	82.74 ± 4.13[b]	87.14 ± 4.35[b]

Values were expressed as mean ± SD for six rats each.

[a] Compared with group I (p<0.001) [b] Compared with group II (p<0.001)

Table 2 shows the effect of of *P. guajava* leaves extract on HDL, VLDL, and LDL- cholesterol in experimental rats

Treatment Groups	HDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)
Group I	24.57 ± 1.22	28.85 ± 1.44	37.54± 1.87
Group II	18.03 ± 0.98[a]	37.71 ± 1.88 [a]	180.26 ± 8.18 [a]
Group III	27.11 ± 1.35[b]	16.57 ± 0.62[b]	37.76 ± 0.08[b]
Group IV (S)	25.86 ± 1.29[b]	17.42 ± 0.57[b]	43.86 ± 2.19[b]

Values were expressed as mean ± SD for six rats each.

[a] Compared with group I (p<0.001) [b] Compared with group II (p<0.001)

DISCUSSION

Streptozotocin (STZ) (2-deoxy-2-(3-methyl-3-nitrosuuredio)-D-glucopyranose) is commonly used for experimental induction of type-I diabetes mellitus, which causes selective pancreatic islet β-cell cytotoxicity mediated through the release of nitric oxide (NO).This results in rapid reduction in pancreatic islet pyridine nucleotide concentration and subsequent β -cell necrosis. The action of STZ on mitochondria generates SOD anions, which leads to diabetic complications. Based on the above perspectives, in the present study, the antidiabetic activity has been assessed in rats made diabetic by STZ. Sulfonylureas such as glibenclamide are often used as a standard antidiabetic drug in STZ-induced diabetes to compare the efficacy of variety of antihyperglycemic compounds [12].In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels [13].

Administration of G1, G2, G3, G4, G5 and G6 to diabetic rats restored the levels of glucose. Present finding is in agreement with Subramaniam et al [14] studies.

Diabetes affects both glucose and lipid metabolism [15]. The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes [16].

The lipoprotein levels in the STZ induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats [17]. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver [18,19]The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx.

The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atheroselerosis [20]. Supplementations of *P. guajava* to diabetic rats restored the lipid profile. Our results concord with the earlier work done by Kesari [21], where it has been reported that lipid profile level in the plasma is restored with the treatment of *Aegle marmelos* seed extract in diabetic rats.

The blood glucose level of *P. guajava* extracts fed animal was significantly (*P*<.001) reduced. The levels of serum TC, TG, LDL, and VLDL were found to be significantly reduced in the plant extracts treated diabetic

animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced lipolysis that might be due to the increase in serum insulin levels in the plant extract treated rats. The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk. In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and α-crystalline of lens [22].

Glycosylated haemoglobin (HbA1) was found to increase in patients with diabetes mellitus to approximately 16% [23] and the amount of increase is directly proportional to the blood glucose level [24]. During diabetes the excess glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin. Therefore, the total haemoglobin level is decreased in STZ diabetic rats. So the total haemoglobin level is lowered in alloxan diabetic rats [25]. Administration of *P. guajava* reversed the total haemoglobin levels in alloxan diabetic rats. The present study suggests that the *P. guajava* extracts had synergetic hypoglycemic effect revealed by decreased serum lipid levels, restored heamoglobin and therefore attribute to therapeutic value of the plant extracts of *P. guajava* to combat the diabetic condition in rats.

CONCLUSION

The results of the present investigation clearly indicate that the *P. guajava* leaves extract shows a better activity to possess possible usefulness in the treatment of diabetes. *P. guajava* leaves extract was found to be more effective in lowering hyperglycemic as well as extremely reduced hyperlipidemic activity. Hence, a great deal of our research gives a solid scientific approach to the traditional uses of these medicinal species effects against diabetes mellitus.

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