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EVALUATION OF ANTI-DIABETIC ACTIVITY ON *GUAVA* (*PSIDIUM GUAJAVA* LINN.) SEEDS AQUEOUS EXTRACT IN *STREPTOZOTOCIN*-INDUCED DIABETIC RATS

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ABSTRACT
Guava (*Psidium guajava* Linn.) commonly known for its food and nutritional values throughout the world. The medicinal properties of guava fruit, leaf and other parts of the plant are also well known in traditional system. In view of suggested anti diabetic potential, effect of aqueous and cold extracts of *Psidium guajava* (Myrtaceae) seeds, on fasting blood sugar levels and serum biochemical analysis in streptozotocin-induced diabetic rats was investigated. All the extracts of *Psidium guajava* produced a significant anti diabetic activity at dose levels of 1/5th of their lethal doses.

KEYWORDS: *Psidium guajava, Anti-diabetic activity, streptozotocin, Aqueous extract, Cold extract.*

INTRODUCTION
Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by ineffectiveness of insulin produced, such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's systems in particular the blood vessels and nerves. As the number of the people with diabetes multiplies worldwide, the disease has taken an ever-increasing share of national and international health care budgets. It is projected to become of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two-to-three- folds campened with the present rates. 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 presentation. Guava is a small tropical tree that grows up to 35 feet tall; it is widely grown for its fruit in tropics. It is a member of the Myrtaceae family, with about 133 genera and more than 3,800 species. The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi *et al.*, 2008). In the view of the immense medicinal importance of *P. Guajava* plant evidenced in there is a strong incentive for further research into the pharmacological activities of *P. guajava* plant extract against common infectious diseases considering the fact that the plant is readily available in the tropics and within the reach of the local populace. Guava contains broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, proteins, sesquiterpenoid alcohols and triterpenoid acids, alkaloids, glycosides, steroids, flavanoids, tannins, saponins, *Psidium guajava* or guava is very rich in antioxidants and vitamins and also high in lutein, zeaxanthine and lycopene. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre and fatty acids. Guava fruit is higher in vitamin C than citrus (80 mg of vitamin C in 100 g of fruit) and contains appreciable amounts of vitamin A as well. Guava fruits are also a good source of pectin - a dietary fiber. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids. The flavonoids have demonstrated antibacterial activity.[1] Quercetin is thought to contribute to the anti diarrheal effect of guava; it is able to relax intestinal smooth muscle and inhibit

bowel contractions. In the light of the above information the present in-vestigation was undertaken to evaluate the anti-diabetic potential of *Psidium guajava*. Seeds extracts on fasting blood sugar and serum biochemical analysis.

MATERIAL AND METHODS

Plant Material: Seeds were collected from ripen fruit of *Psidium guajava* in local area market near mahabuabad. Telanagana, india.

Preparation of Extracts: The seeds were shade dried at room temperature and subjected to size reduction to coarse powder by using dry grinder and passed through sieve no 40.

Aqueous extraction: The powder of *Psidium guajava seeds* were packed in a Soxhlet apparatus and extracted with distilled water for 18 hours. The obtained extract (7.5%) was dried at 45°C in hot air oven till solid/semisolid mass was obtained.

Cold extraction: Approximately about 100 g of the powdered seeds is taken in a 2000 ml conical flask with 500 ml of distilled water and 10 ml of chloroform added as a preservative (maceration process). It was extracted up to one week with daily 2 hours stirring with a mechanical stirrer. After 7 days the extract was filtered through muslin cloth and marc was discarded and its filtrate (8.2%) dried in a hot air oven at 45°C till solid/semisolid mass, was produced. Both the extracts were stored in air tight container in refrigerator below 10°C. The suspensions of aqueous and cold extracts were prepared by using normal saline as solvent for administration to experimental animals.

Animals Used: Wistar albino rats (150-200 g) and Wistar albino mice (20-25 g) of both sexes were procured from Indian Institute of Sciences, Bangalore, India. Before and during the experiment rats were fed with standard diet (Gold Mohr, Lipton India Ltd). After randomization to various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. Animals described as fasting were deprived of food and water for 16 hours *ad libitum*.

Sample Collection

Blood samples were collected by the retro-orbital plexus puncture method and blood glucose levels were estimated using an electronic glucometer (Miles Inc., USA) and glucostix (Bayer diagnostic India Ltd., Baroda).

Experimental Design

All the animals were randomly divided in the five groups with six in each group. Group I, II and III were administered saline, diabetic, and standard drug (glibenclamide, 10 mg/kg per day p.o) control, respectively. Preliminary oral LD50 doses of aqueous and cold extract of *Psidium guajava in* mice were found to be 215, 230 mg/kg respectively. Group IV and V were treated with seed extracts in one-fifth of LD50 doses of aqueous extract (43g/kg per day p.o) and cold extract (46 mg/kg per day p.o), respectively.

Assessment of Extracts on streptozotocin -Induced Diabetic Animals

Rats were made diabetic by a single intraperitoneal injection of 150 mg/kg)[2] [12] *streptozotocin* was first weighed individually for each animals according to the weight and solubilized with 0.2ml saline (154 mM NaCl) just prior to injection. Two days after *streptozotocin* injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 hours after *streptozotocin* injection. Blood sample were drawn at weekly intervals till end of study (i.e. 3 weeks). Fasting blood glucose estimation and body weight measurement were done on day of 1, 7 and 21 of the study. On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated.[3] Serum was separated and analyzed for serum cholesterol[4], se-rum triglycerides by enzymatic DHBS colorimetric method[5], serum HDL[6], serum LDL[7], serum creatinine[8], serum urea[9] and serum alkaline phosphatase by hydrolyzed phenol amino antipyrine method.[10]

Statistical Analysis

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (SEM) and analyzed using Student‘t’ test.

RESULTS

The antidiabetic effects of the extracts on the fasting blood sugar levels of diabetic one shown in Fig 1. Administration of *streptozotocin* (150 mg/kg, i.p) led to 1.5-fold elevation of fasting blood glucose levels, which was maintained for period of 3 weeks. Three weeks of daily treatment of extracts led to a dose-dependent fall in blood sugar levels by 25-62%. Effect seems to reach maximum after 15 days of treatment and remained constant in third week. Vehicle control animals were found to be stable in their body weight while diabetic rats showed significant reduction in body weight during 21 days (Table 1). *streptozotocin* caused weight reduction, which was reversed by aqueous and cold extracts of *Psidium guajava* after 7 days of treatment. Serum cholesterol, serum triglycerides, serum LDL, serum creatinine, serum urea, and serum alkaline phosphatase levels were decreased significantly by glibencalmide ($p < 0.001$), aqueous extract ($p < 0.001$) and cold extract ($p < 0.01$) of *Psidium guajava*, after 21 days of treatment compared with diabetic control. HDL levels were increased by glibenclamide ($p < 0.001$), aqueous extract ($p < 0.001$) and cold extract ($p < 0.01$) compared with diabetic control (Table 2).

Table 1: The effect of 3-week treatment with Aqueous and cold extracts of *Psidium guajava* . on body weight (g) after *streptozotocin* (150 mg/kg i.p.) induced diabetes in rats.

Group No	Treatment	Dose (mg/kg P.O)	Average body weight (g) ± SEM			
			Day 1	Day 7	Day 14	Day 21
I	Vehicle control	0.2 ml a	200.1 ± 1.9	201.83 ± 1.02	203.00 ± 1.05	205.83 ± 1.52
II	Diabetic control	0.2 ml b	206.2 ± 2.1	176.00 ± 5.2	162.33 ± 2.51	148.83 ± 1.62
III	Glibenclamide	10	206.8 ± 2.2	198.00 ± 1.31**	195.21 ± 2.33***	192.00 ± 3.96***
IV	Aqueous extract	43	207.3± 2.07	196.16 ± 1.70*	190.21 ± 1.72**	181.22 ± 4.3**
V	Cold extract	46	206.9 ± 1.83	197.02 ± 2.2*	189.72 ± 3.1**	180.47 ± 5.2**

Values are given in average body weight (g) ±SEM for groups of six animals each.
a Normal saline.
b Normal saline + *streptozotocin*.
Significance vs. control group.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

Table 2: Effect of aqueous and cold seed extract of *Psidium guajava* on serum profile in *streptozotocin* (150 mg/kg, i.p.) induced diabetic albino rats after 21 days of treatment.

Group No.	Treatment	Dose (mg/kg P.O)	Serum cholesterol	Serum triglycerides	Serum HDLcholesterol	Serum LDLcholesterol	Serum creatinine	Serum urea	Serum alkalinephosphatase
I	Vehicle control	0.2 ml a	151.00±6.2	86.83±5.5	37.00±1.5	93.23±5.5	0.52±0.1	24.66±1.00	115.16±2.0
II	Diabetic control	0.2 ml b	269.33±15.5	200.83±11.1	29.00±1.1	199.16±14.2	1.35±0.1	61.00±1.1	304.50±5.1
III	Glibenclamide	10	146.83±6.1***	108.00±6.1***	50.50±1.8***	73.73±6.7***	0.58±0.1***	32.00±2.2***	131.16±4.1***
IV	Aqueous extract	43	154.83±3.8***	115±6.1***	39.83±1.5***	94.56±2.7**	0.63±0.1***	31.03±1.2***	134.66±5.1***
V	Cold extract	46	162.8±4.1**	122±6.1**	43.22±1.5**	101.02±3.5**	0.72±0.1**	34.22±1.1**	136.22±4.1**

Values are given in average body weight (g) ±SEM for groups of six animals each.
a Normal saline.
b Normal saline + *streptozotocin*.
Significance vs. control group.
* $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$.

DISCUSSION AND CONCLUSION

In the light of the results, our study indicates that *Psidium guajava seeds* extracts have anti-diabetic activity. Aqueous and cold extracts of *Psidium guajava* exhibited significant anti- hyperglycemic activities in *streptozotocin*-induced hyperglycemic rats without significant change in body weight. They also improved conditions of DM as indicated by parameters like bodyweight, and lipid profiles along with serum]. In *streptozotocin*-induced diabetes, (-)-epicatechin [creatinine, serum urea, and serum alkaline phosphatase. The number of functionally intact β-cells in the islet organ is of decisive importance the development course and outcome of DM. The renewal of β-cells in diabetes has been studied in several animal models. The total β-cell mass reflects the balance between the renewal and loss of these cells. It was also suggested that regeneration of islet β-cells following destruction by *streptozotocin* may be the primary cause of the recovery of *streptozotocin* –injected guinea pigs from the effects of the drug.

This effect may be due to β-carotene, which was reported to be constituents of *Psidium guajava*.[11] The benefi-cial role of β-carotene in reducing diabetic complications like glycosylation in *streptozotocin*-induced diabetic rats[12] had been reported previously. Photomicrographical data in our studies confine healing of pancreas by *Psidium guajava seeds* extracts, as a plausible mechanism of their anti diabetic activity.

Aqueous and cold extract of *Psidium guajava seed* exhibited significant anti hyperglycemic activities in *streptozotocin*-induced diabetic rats. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of β-cells of pancreas and so might be of value in diabetes treatment.

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