ANTIHYPERGLYCAEMIC ACTIVITY OF ETHANOL EXTRACT OF *PSIDIUM GUAJAVA* LEAVES IN ALLOXAN INDUCED DIABETIC MICE.

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Summary

The antihyperglycaemic activity of the ethanol extract of *Psidium guajava* (EEPG) leaves was studied by administering three doses of EEPG (i. e.100,200 and 400 mg/kg, p. o.) to alloxan (70 mg/kg, i. v.) induced diabetic mice. The serum glucose levels and body weights of mice were determined. The acute oral toxicity study showed no mortality upto 5000 mg/kg p. o. dose of EEPG. In acute study the antihyperglycaemic effect was observed at 2h (78.25 mg/dl), peak at 6 h (197.03 mg/dl) but antihyperglycaemic effect vanished at 24 h. The subacute study was also carried out which showed maximum reduction in serum glucose level (311.22 mg/dl) at the dose of 200 mg/kg on 28th day. The oral glucose tolerance test (OGTT) was carried out after administration of EEPG in non-diabetic and diabetic mice previously loaded with (2.5 g/kg, p. o.) glucose. EEPG (200 mg/kg) showed increased glucose threshold in non-diabetic mice. EEPG (800 mg/kg) showed increased glucose threshold in diabetic mice. EEPG (200 mg/kg) prevented the loss of body weight. These results indicated antihyperglycaemic activity of EEPG (200 mg/kg) in alloxan induced diabetic mice. The antihyperglycaemic activity of EEPG was comparable with glyburide.

Keywords: *Psidium guajava*, Alloxan, Antihyperglycaemic, Body weight, Oral glucose tolerance test.

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Introduction

Since ancient times, several medicinal plants, their extracts or different formulations have been used in the treatment of diabetes mellitus. The main advantages of herbal medicines are their inherent efficacy, low incidence of side effects and low cost. Though many plant products have been proved to be useful in the control of diabetes mellitus, none of them has been emerged as a potent remedy for this disorder (1). Hence emphasis is on the development of drugs from plants for the treatment of diabetes mellitus, the incidence of which is very high all over the world especially in India.

Psidium guajava Linn. (Myrtaceae) is commonly known as 'Peru' or 'Jamba' or 'Jam' in Marathi, 'Amrud' in Hindi and 'Guava' in English. It is cultivated and naturalized throughout India and most tropical countries (2). It is small tree up to 8m tall with smooth, peeling bark. Leaves are short petioled, elliptic-ovate to elliptic oblong, often rounded at base, leathery, pubescent on lower surface, 15cm long and 6cm wide, lateral nerve 10 -18 pairs prominent on undersurface. Flowers large, white and fragrant, 2cm long. Fruit 3-10 cm across, green to light yellow when ripe (3).

The leaves of *Psidium guajava* are used in the treatment of diarrhea, cough, stomachache, dysentery and decoction of the leaves for cholera patients, toothache and gum boils. The leaves showed hypoglycemic, cardioprotective, myocardial depressant, antimicrobial, antispasmodic actions (4). Fruits and leaves are used as antidiabetic (5). The objective of present investigation was to study the antihyperglycaemic activity of Ethanolic Extract of Psidium guajava leaves in alloxan induced diabetic mice.

Materials and Methods

Collection and authentication of plant

The leaves of *P. guajava* were collected from the local area of Pune in Maharastra state and were authenticated by Dr. A.M. Mujumdar, Department of Botany, at Agharkar Research Institute,
Pune and voucher specimen was deposited at that Institute as Voucher No. AHMA L-02 on 29-07-2006.

Drugs and Chemicals
Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and d-glucose (S.D. Fine-Chem. Ltd, India) and ethanol (Merck, Mumbai, India) were purchased from respective vendors.

Extraction and preparation of EEPG
The leaves of *P. guajava* were dried in shade and powdered in grinder. The air dried powder was subjected to hot continuous extraction with ethanol in a soxhlet extractor and filtered. The filtrate was evaporated at room temperature and the extract concentrated on a water bath to dry residue. The % yield of ethanol extract was 4.27 % w/w. The EEPG was dissolved in distilled water to prepare the drug solution of concentration of 100 mg/ml and used for pharmacological studies.

Experimental animals
Swiss albino mice (25-30 g) of either sex were purchased from the National Toxicology Centre, Pune, India. Animals were housed under standard condition of temperature 25 ± 1^0C and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India), and water was given *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Poona College of Pharmacy, Pune, India.

Acute oral toxicity studies
Healthy adult Swiss albino mice of female sex weighing between 18 to 23 g were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development (6). The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles for any lethality or death for the next 48 h.

Induction of experimental diabetes
Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg /kg i.v.). After 48 h, the animals showing serum glucose levels above 200 mg /dl (diabetic) were selected for the study (7). All the animals were allowed free access to water and pellet diet.

Collection of blood and determination of serum glucose
Blood samples were collected by retro-orbital puncture (ROP) technique. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method (8) and serum glucose levels were expressed in mg/dl.

Effect of EEPG on serum glucose in alloxan-induced diabetic mice
The diabetic mice of either sex were divided into five groups (n =6), viz.: group I-vehicle (distilled water, 10 ml/kg); group II-glyburide (10 mg/kg); group III-EEPG (100 mg/kg); group IV- EEPG (200 mg/kg); group V- EEPG (400 mg/kg). All drugs were given orally. The acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after drug administration (9).

The subacute study involved repeated administration of drug for 28 days at prefixed times and serum glucose levels were estimated on the 7th, 14th, 21st and 28th days. At the end of 28 days the drug administration was stopped and a rest period of 7 days was given to the animals to study effect of drug treatment on blood glucose after 7 days i.e. on 35th days. The data were represented as mean serum glucose level and standard error of mean (SEM). The mice were weighed daily during the study period of 35 days, and their body weights were noted and presented as mean change in body weights.

Effect of EEPG on oral glucose tolerance test (OGTT) in non-diabetic and diabetic mice
The animals were fasted overnight before commencing the experiment. Diabetic mice were divided into five groups (n = 6), viz.: group I-d-glucose (2.5 g/kg); group II-glyburide (10 mg/kg); group III- EEPG (200 mg/kg); group IV- EEPG (400 mg/kg); group V- EEPG (800 mg/kg).

Non-diabetic mice were divided into three groups (n = 6), viz.: group I-d-glucose (2.5 g/kg); group II-glyburide (10 mg/kg); group III- EEPG (200 mg/kg). D-glucose (2.5 g/kg) was administered in non-diabetic and diabetic mice at the 4^{th} h of pretreatment with EEPG and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading.

Statistical analysis
The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by *post hoc* Tukey's test (10).

Results

In acute oral toxicity study, EEPG was safe upto a dose level of 5000 mg/kg of body weight. No lethality or any toxic reactions were found upto the end of the study period.

In acute study, EEPG (100,200 and 400 mg/kg) as well as glyburide (10 mg/kg) showed significant reduction of serum glucose levels at 2, 4, and 6 h. The onset of reduction of serum glucose of EEPG (100, 200 and 400 mg/kg) was observed at 2 h (80.03, 78.25 and 49.11 mg/dl respectively), peak effect at 6 h (181.60, 197.03 and 141.25 mg/dl respectively) but effect was waned at 24 h. The onset of antihyperglycaemic effect of glyburide was at 2 h (94.04 mg/dl), the peak effect was at 6 h (237.90 mg/dl) (Table 1).

Table 1: Effect of EEPG on serum glucose level in alloxan-induced diabetic mice (acute study).

Groups (Treatment mg/kg)	Mean Fasting Serum Glucose Level (mg/ dl) ± SEM				
	0 h	2 h	4 h	6 h	24 h
Group I (Vehicle)	437.5 ± 9.66	443.74 ± 12.58	447.60 ± 13.26	452.40 ± 16.58	461.55 ± 12.67
Group II (Glyburide 10)	446.83 ± 12.63***	352.79 ± 11.89***	284.24 ± 11.43***	208.93 ± 18.90***	330.09 ± 20.45***
Group III (EEPG 100)	458.00 ± 11.46	377.97 ± 11.26*	349.51 ± 15.15***	276.40 ± 23.12***	369.83 ± 22.83**
Group IV (EEPG 200)	447.33 ± 14.03	369.08 ± 13.87**	334.75 ± 12.78***	250.30 ± 15.27***	348.40 ± 13.50***
Group V (EEPG 400)	439.33 ± 13.24	390.22 ± 14.89ns	357.54 ± 14.25**	298.08 ± 14.46***	384.27 ± 16.87*

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

In the subacute study, repeated administration (once a day for 28 days) of the EEPG as well as glyburide caused significantly ($P < 0.001$) reduction in the serum glucose level as compared with vehicle treated group. Maximum reduction in serum glucose level was observed (311.22 mg/dl) on 28th day in the diabetic mice treated with EEPG (200 mg/kg). Maximum reduction in serum glucose level was observed (309.73 and 251.35 mg/dl respectively) on 35th day in the diabetic mice treated with EEPG (100 and 400 mg/kg). Glyburide treated animals showed maximum reduction in serum glucose level (312.28 mg/dl) on 21st day (Table 2).

Table 2: Effect of EEPG on serum glucose level in alloxan-induced diabetic mice (subacute study).

Groups (Treatment mg/kg)	Mean Fasting Serum Glucose Level (mg/ dl) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I (Vehicle)	437.50 ± 9.66	469.48 ± 14.67	476.93 ± 14.81	484.01 ± 15.59	491.67 ± 15.91	499.67 ± 15.72
Group II (Glyburide 10)	446.83 ± 12.63^{ns}	247.23 ± 17.56	213.42 ± 16.70	134.55 ± 20.02	163.11 ± 19.59	186.05 ± 19.21
Group III (EEPG 100)	458.00 ± 11.46^{ns}	316.20 ± 20.13	277.98 ± 17.67	228.42 ± 21.42	178.31 ± 21.51	148.27 ± 22.09
Group IV (EEPG 200)	447.33 ± 14.03^{ns}	280.36 ± 13.88	232.93 ± 13.57	183.52 ± 14.82	136.11 ± 16.52	177.97 ± 17.45
Group V (EEPG 400)	439.33 ± 13.24^{ns}	368.36 ± 17.30**	355.21 ± 20.11	274.95 ± 17.61	238.71 ± 17.55	187.98 ± 18.38

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, ns- not significant, **P<0.01.All other values are significant (P<0.001) as compared with vehicle-treated group (distilled water, 10 ml/kg).

Body weight of vehicle and EEPG (100 and 400 mg/kg) treated diabetic mice decreased during study period. EEPG (200 mg/kg) and glyburide (10 mg/kg) prevented further loss of body weight in diabetic mice. On the other hand, mice gained body weight which indicated beneficial effect of EEPG. (Table 3).

Table 3: Effect of EEPG on body weight in alloxan-induced diabetic mice.

Groups (Treatment mg/kg)	Mean Body Weight (g) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Group I Vehicle	31.33 ± 1.14	28.33 ± 1.05	26.50 ± 0.99	25.00 ± 1.06	23.50 ± 1.11	22.33 ± 1.14
Group II (Glyburide 10)	34.16 ± 0.94	34.50 ± 0.61**	34.50 ± 1.20***	34.83 ± 0.90***	34.83 ± 1.13***	35.16 ± 1.19***
Group III (EEPG 100)	30.16 ± 0.90	29.33 ± 0.95	28.50 ± 0.95	26.83 ± 0.94	24.00 ± 1.03	24.83 ± 0.94
Group IV (EEPG 200)	33.00 ± 0.93	33.50 ± 0.84*	34.00 ± 1.15	34.66 ± 1.08***	35.00 ± 1.03***	35.00 ± 1.18***
Group V (EEPG 400)	29.00 ± 1.67	28.16 ± 1.13	26.83 ± 1.22	25.00 ± 1.21	23.50 ± 1.20	25.16 ± 1.30

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

In oral glucose tolerance test, EEPG (200 mg/kg) and EEPG (800 mg/kg) produced significant (P<0.001) increase in glucose threshold, 4^{th} h post glucose loading in non-diabetic (Table 4) as well as diabetic (Table 5) mice respectively. These results suggest that EEPG possessed antihyperglycaemic activity in alloxan induced diabetic mice.

Table 4: Effect of EEPG on oral glucose tolerance test (OGTT) in non-diabetic mice.

Groups (Treatment mg/kg)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM		
	0 h	Before glucose load	After glucose load (6 h)
Group I (Vehicle)	108.66 ± 2.65	117.88 ± 2.60	293.14 ± 4.97
Group II (Glyburide 10)	107.50 ± 4.76	114.40 ± 4.82	151.85± 9.96***
Group III (EEPG 200)	90 .31 ± 5.27	100.88 ± 9.40	119.71± 5.57***

D-glucose (2.5 g/kg) was administered in non-diabetic mice at the 4th h of pretreatment with EEPG and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading.
Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

Discussion

Psidium guajava leaves and fruits are used in medicine for the treatment of diabetes. The leaf extract inhibited hyperglycaemia in alloxan induced diabetic rats (11). The hypoglycaemic properties of water extract of the Psidium guajava leaves have been reported (12).

EEPG (100, 200 and 400 mg/kg) showed significant (P<0.001) decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with EEPG (100, 200 and 400 mg/kg) for a period of 35 days showed a significant (P<0.001) decrease in the serum glucose level in diabetic mice. Maximum reduction of serum glucose level in acute and subacute occurred at the dose of 200 mg/kg. p. o. The EEPG showed short onset and short duration of antihyperglycaemic action.

Table 5: Effect of EEPG on oral glucose tolerance test (OGTT) in diabetic mice.

Groups (Treatment mg/kg)	Mean Fasting Serum Glucose Level (mg/dl) ± SEM)		
	0 h	Before glucose load	After glucose load (6 h)
Group I (Vehicle)	419.53 ± 4.98	420.86 ± 5.36	523.93 ± 5.84
Group II (Glyburide 10)	417.44 ± 9.45	424.23 ± 10.94	357.19 ± 13.02***
Group III (EEPG 200)	455.32 ± 19.82	447.78 ± 16.42	409.53 ± 12.64***
Group IV (EEPG 400)	449.08 ± 17.55	383.85 ± 27.12	318.74 ± 19.34***
Group V (EEPG 800)	438.89 ± 12.37	347.05 ± 13.69*	272.37 ± 15.62***

D-glucose (2.5 g/kg) was administered in diabetic mice at the 4th h of pretreatment with EEPG and glyburide. Serum glucose levels were estimated before and 2 h after glucose loading.
Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one- way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, ***P<0.001 as compared with vehicle-treated group (distilled water, 10 ml/kg).

Subacute treatment for 35 days with the EEPG in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic mice (13). The ability of EEPG to prevent body weight loss seems to be due to its ability to reduce hyperglycaemia.

OGTT study indicated that EEPG enhanced glucose utilization in non- diabetic & diabetic mice. Administration of EEPG effectively prevented the increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. In this context, other medicinal plants, such as *Pleurotus pulmonarius* (9), *Cassia auriculata* (14), *Ficus racemosa* (15), *Ficus religiosa* (16) have been reported to possess similar effect.

Flavonoids are potent antioxidants and known to modulate the activities of various enzymes due to their interaction with various bio molecules (17). Kameswararao et al (1997) reported that flavonoids, alkaloids, tannins & phenolics as bioactive antidiabetic principles (18).

The plants of *Psidium guajava* have been reported to contain myrcene, limonene, β-caryophyllene, farnesene, β-humulene, β-bisabolene, β-sitosterol, α-pinene, β-pinene, ursolic acid, α and β-selinene, cadinene and curcumene (4), isostrictinine, strictinine, pedunculagin, three new tannins-guavine A, C and D (11). Preliminary phytochemical analysis indicated that the leaf extract of *Psidium guajava* contain sterols, triterpenoids, glycosides, tannins and carbohydrates.

The antihyperglycaemic activity of EEPG may probably be due to presence of several bioactive antidiabetic principles. It is thus apparent that EEPG possesses antihyperglycaemic activity. The further study of isolation of active constituent by TLC and flash chromatography and antihyperglycaemic activity of isolated constituent is ongoing.

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