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Antidiabetic activity of an ethanol extract obtained from the stem bark of *Psidium guajava* (Myrtaceae)

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This study analyzed the antidiabetic properties of an ethanol extract of the stem bark of *Psidium guajava*, an indigenous medicinal plant used to control diabetes in Indian System of Medicine. The antihyperglycaemic activity of this plant on blood glucose levels of normal, normal glucose loaded (OGTT) and alloxan-induced hyperglycaemic rats was evaluated. The results showed that ethanol stem bark extract exhibited statistically significant hypoglycaemic activity in alloxan-induced hyperglycaemic rats but was devoid of significant hypoglycaemic effect in normal and normal glucose loaded rats (OGTT).

1. Introduction

Psidium guajava Linn. (Myrtaceae), an arborescent shrub or small tree, up to 8 m high, is native of tropical America and has long been naturalized in India (Anonymous 1998). The leaves contain catechol, tannins, wax, resins, sugars, carotenoids (Mercadante et al. 1999), vitamins A, B_1, B_2, B_6, niacin (Sharma et al. 1999; Anonymous 1998). The essential oil contains alpha-pinene, 1,8-cineole (Xadio et al. 1991), alpha selinene, beta-caryophyllene and delta-selinine (Sagrero-Nieves et al. 1994; Ambasta 1986) as the major components. From the bark six new complex tannins, guajavins A and B, psidium A, B and psiguanin together with a variety of condensed, hydrolysable and complex tannins have been isolated (Tanaka et al. 1992; Rastogi and Mehrotra 1991, 1993), calcium and manganese in combination with phosphoric, oxalic and maleic acids (Nadkarni 1985) have also been reported. Volatile constituents from the fruits include beta-caryophylene, limonene, 3-phenyl propyl acetate, (*Z*)-3-hexenyl acetate, (*E*)-cinnamyl acetate (Pino et al. 1999). The leaves are used as astringent, anodyne, febrifuge, antispasmodic, tonic, in wounds, cholera, diarrhoea, vomiting (Vaidyaratnam 1995; Anonymous 1998), for swollen gums and ulceration of mouth. The bark is used as astringent, febrifuge and antiseptic (Nadkarni 1985). The roots are used as astringent, haemostatic, antiemetic. The fruits are sweet, astringent, cooling, aphrodisiac, laxative and tonic (Vaidyaratnam 1995; Ambasta 1986). Among the biological activities of the plant antidiarrhoeal (Lutterodt 1992), anticough and antimicrobial (Jairaj et al. 1999), analgesic, anti-inflammatory, CNS depressant (Olajide et al. 1999; Matsunaga et al. 1997; Mecks et al. 1996; Santos et al. 1998; Rabe and Van Steden 1997; Lutete et al. 1994), topical haemostatic (Jairaj et al., 2000), atiamoebic (Tone et al. 1998), antipyretic, antiarthritic (Sen et al. 1995), hypoglycemic (Obatomi et al. 1994; Roman-Ramos et al. 1995) properties have been reported.

2. Investigations, results and discussion

The effect of the ethanol extract of *Psidium guajava* on glucose tolerance is shown in Table 1. By 30 min after starting the oral glucose tolerance test, the blood concentration increased marginally from its initial value of control and then glycemia started to decrease gradually until the end of the study i.e., at the 90th min. The test drug showed a marginal hypoglycemic effect. Gliclazide exerted a statistically significant hypoglycemic effect in normal fasted rats (Table 2).

Table 1: Effect of *P. guajava* bark, ethanol extract (250 mg/kg, p.o.), on oral glucose tolerance in normal rats[a] (OGTT)

Sample time (min)	Blood glucose level (mg/dl)		
	Vehicle (Distilled water)	Gliclazide (25 mg/kg)	Ethanol extract (250 mg/kg)
0	65.00 ± 1.00	64.4 ± 2.17	72.6 ± 0.97
30	96.00 ± 2.30	63.4 ± 2.30***	89.80 ± 2.43^{NS}
90	78.40 ± 2.69	48.0 ± 2.15***	81.80 ± 2.69^{NS}

[a] Values are means ± S.E.; n = 5
*** $p < 0.001$, NS, not significant difference to the vehicle

Table 2: Effect of *P. guajava* bark, ethanol extract (250 mg/kg, p.o.), on blood glucose level in normal rats[a]

Sample time (h)	Blood glucose level (mg/dl)		
	Vehicle (Normal saline)	Gliclazide (25 mg/kg)	Ethanol extract (250 mg/kg)
0	51.60 ± 2.58	53.40 ± 3.14	55.60 ± 1.28
1	66.40 ± 6.04	38.0 ± 2.70	57.20 ± 0.58^{NS}
3	71.0 ± 3.60	40.40 ± 2.52	62.40 ± 1.69^{NS}

Values are means ± S.E.; n = 5, NS, not significant difference to the vehicle

Oral administration of the ethanol extract led to a significant blood glucose lowering effect in alloxan-induced hyperglycemic rats (Table 3). The fall was seen at 1 h and remained upto 3 h after the administration of the extract whereas the fall in case of gliclazide administration was marginal. This may be due to the fact that alloxan treatment causes permanent destruction of the β-cells (Pari and Maheshwari 1999) and gliclazide requires more than 30% functional pancreas for the effect (Zarrow et al. 1964).

During sub-acute treatment of alloxan-induced hyperglycemic rats with *P. guajava* ethanol extract, a consistent reduction in the blood glucose levels was observed (Table 4). Again the hypoglycemia shown by gliclazide was marginal as was evident during the acute treatment.

This study reports the antihyperglycaemic effect of the ethanol extract of *P. guajava* (stem bark), a plant drug used in the traditional system of medicine for the treatment of diabetes. Gliclazide (a sulfonylurea) is known to lower the blood glucose level by stimulating β-cells to release insulin, since alloxan induces hyperglycemia by destroying β-cells (Pari and Maheshwari 1999) and impairing renal function. In the present study, gliclazide exhibited marginal hypoglycemic activity in alloxan-induced hyperglycemic rats, but was devoid of statistically significant anti-diabetic activity in normal and normal-glucose loaded rats. However, in diabetic rats, the ethanol extract showed a considerable and statistically significant hypoglycemic effect, which may not be due to potentiation of insulin release from pancreatic cells, but may be due to extrapancreatic mechanism. As far as the mechanism of action is concerned, we can speculate that the hypoglycaemic activity of *P. guajava* stem bark could be due to an enhancement of peripheral metabolism of glucose. Studies are underway to isolate the active constituent(s) from the ethanol extract and to find out the mechanism of action involved.

Table 3: Effect of acute treatment of *P. guajava* bark, ethanol extract (250 mg/kg, p.o.), on blood glucose level in alloxan induced diabetic rats[a]

Sample time (h)	Blood glucose level (mg/dl)		
	Vehicle (Distilled water)	Gliclazide (25 mg/kg)	Ethanol extract (250 mg/kg)
0	353.80 ± 2.30	329.00 ± 2.57	313.40 ± 3.59
1	352.60 ± 2.13	315.60 ± 2.70^{NS}	206.40 ± 3.45***
3	349.20 ± 2.08	308.20 ± 2.52^{NS}	223.40 ± 2.60***

[a] Values are means ± S.E.; n = 5
*** $p < 0.001$, NS, not significant difference to the vehicle

Table 4: Effect of sub-acute treatment of *P. guajava* bark, ethanol extract (250 mg/kg, p.o., once daily), on blood glucose level in alloxan induced diabetic rats[a]

Sample time (Day)	Blood glucose level (mg/dl)		
	Vehicle (Distilled water)	Gliclazide (25 mg/kg)	Ethanol extract (250 mg/kg)
0	353.80 ± 2.30	329.00 ± 2.57	313.40 ± 3.59
1	352.80 ± 2.13	303.80 ± 2.70^{NS}	223.60 ± 2.50***
3	349.20 ± 2.08	306.40 ± 2.82^{NS}	167.80 ± 2.95***
7	354.40 ± 2.52	308.50 ± 1.89^{NS}	153.00 ± 2.60***
10	356.80 ± 2.06	304.60 ± 2.17^{NS}	138.00 ± 2.75***

[a] Values are means ± S.E.; n = 5
*** $p < 0.001$, NS, not significant difference to the vehicle

3. Experimental

3.1. Plant material

Fresh stem bark of *P. guajava* was collected from the Bullandshehar district of Uttar Pradesh, India and authenticated at the Taxonomy Division, Department of Botany, Faculty of Science. A voucher specimen (PG-FP-03) was deposited in the Laboratory of Pharmacognosy and Phytochemistry.

3.2. Preparation of the ethanol extract

Shade dried, powdered bark (3 kg) was extracted with ethanol (95%) and filtered. The filtrate was dried by vacuum rotary evaporation, which yielded a crude residue of 36.5 g.

3.3. Pharmacological analysis

3.3.1. Test animals

Wistar rats (180–200 g) of either sex were used. They were procured from the Central Animal House, Jamia Hamdard, New Delhi (173/CPCSEA), after approval under project number 135 and housed under standard environmental conditions of temperature, relative humidity and dark and light cycle at the animal house. They were fed standard diet (Hindustan Lever, India). The animals were fasted for 16 h prior to experiment, with water *ad libitum*.

3.3.2. Studies in normal glucose loaded animals (OGTT)

Fasted normal rats were divided into three groups of five animals each. Group I served as control and received distilled water. Group II received the standard drug, gliclazide at an oral dose of 25 mg/kg and Group III received ethanol extract at an oral dose of 250 mg/kg. 30 minutes after extract administration, the rats of all the groups were orally treated with 2 g/kg of glucose (Joy and Kuttan 1999; Venkatesh et al. 2003). Blood samples were collected from the tip of the tail just prior to glucose administration and 30, and 90 min after glucose loading. Serum was separated and blood glucose levels were measured immediately by the glucose oxidase method (Varley et al. 1980).

3.3.3. Effect of test drug on blood glucose levels in normal fasted rats

Fasted rats were divided into three groups of five animals each. Group I served as control and received only vehicle (distilled water) orally. Group II served as standard and received the standard drug, gliclazide at an oral dose of 25 mg/kg and Group III served as test and received the ethanol extract at an oral dose of 250 mg/kg.

3.3.4. Induction of experimental hyperglycemia

Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate (s.d. fine-chem. Ltd., Mumbai, India) in sterile saline (Joy and Kuttan 1999). After 5 days of alloxan injection, the hyperglycemic rats (glucose level >300 mg/dl) were separated and used for the study.

3.3.5. Effect of P. guajava ethanol extract on alloxan-induced hyperglycemic rats

Acute treatment: The hyperglycemic rats were divided into three groups of five animals each. Group I was previously selected from normal rats and served as a normal control and received distilled water only and no alloxan. Group II served as diabetic control and received distilled water. Group III received standard anti diabetic drug gliclazide at an oral dose of 25 mg/kg (Panacea Biotech Ltd., Batch No. 01030513). Group IV was treated orally with ethanol extract at a dose of 250 mg/kg. Blood samples were collected from the tip of tail just prior to and 1 and 3 h after the extract/standard drug administration.

Sub-acute treatment: In sub-acute treatment, the administration of extract/standard drug was continued for 10 days, once daily. Blood samples were collected from the tip of the tail just prior to and on days 1, 3, 7 and 10 after the extract/standard drug administration and the blood glucose levels were determined immediately.

3.4. Statistical analysis

The results are presented as means as ± SEM. The statistical significance among groups was first analyzed by ANOVA, followed by Dennett's comparison test to check significance between groups. P-values of less than 0.05 were considered significant.

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