HYPOGLYCAEMIC AND ANTIDIABETIC ACTIVITY OF *TEPHROSIA PURPUREA (LINN)* ROOT EXTRACTS

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Summary

The present study was undertaken to evaluate the hypoglycaemic and antidiabetic activity of root extracts of *Tephrosia purpurea (Fabaceae).* In traditional literature, it is found that roots of *Tephrosia purpurea* were used as folk medicine for the treatment of Diabetes mellitus in different parts of the world. Roots of crude drug (*Tephrosia purpurea*) extracted successively using various solvents [Pet-Ether (60-80^{0}C), 95% ethanol and aqueous alcohol (60% water + 40% ethanol). The extracts are screened for their possible hypoglycemic and antidiabetic activity. Hypoglycaemic activity was evaluated with normal healthy Wistar rats while antidiabetic activity was evaluated with alloxan induced diabetic rats. In preliminary phytochemical investigations Petroleum ether extract showed presence of Phytosterols; alcoholic extract showed positive tests to tannins and glycoside and hydro-alcoholic extract revealed presence of tannins. Single dose administration of all the extracts of *Tephrosia purpurea* did not showed any hypoglycemic or anti-diabetic activity. Repeated dose administration of alcoholic and hydro-alcoholic extract showed significant hypoglycemic and antidiabetic activity at the end of 7th day. These results suggest that aqueous and hydro-alcoholic extract possess antidiabetic activity.

Keywords: *Tephrosia purpurea*, hypoglycemic activity, Antidiabetic activity

Introduction

'Diabetes' meaning Siphon was coined by the Greek physician Arateus in the second century. He described patients with great thirst (polydypsia) and excessive urination (polyurea). In the 17th century, it was observed that urine of diabetic patient was sweet, so the word mellitus (honey) added to it. Diabetes mellitus also known as 'Madhumeha' in Ayurveda. Diabetes mellitus is also known as rich man disease because people who are over nourished usually get affected by diabetes.[1]

Diabetes is a chronic incurable condition[2] or described as heterogenous group of disorders charaterized by varying degrees of insulin hyposecrection and or insulin insensitivity. Regardless of cause, it is associated with hyperglycemia.[3]

Tephrosia purpurea is a widely growing small plant belonging *Fabaceae* family. The roots, leaves, seeds, and bark were used medicinally[4]. The herb has white to purplish flowers and can be found in tropical regions. According to ayurveda, *Tephrosia purpurea* is used as digestible, anthelmintics, alexiteric, antipyretic, astringent, thermogenic, acrid and also used to cure diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy and asthma.[5] Unani system of medicine describes the roots as diuretic, allays thirst, enriches blood, cures diarrhea, useful in bronchitis, liver, inflammations, boils and pimples.[6,7] *Tephrosia purpurea* is reported to contain three novel flavonoids, (+)-tephrorins A and B and (+)-tephrosone[8]. An isoflavone, 7,4'-dihydroxy-3',5'-dimethoxyisoflavone (1), and a chalcone, (+)-tephropurpurin (2), both novel compounds, as well as six constituents of known structure, (+)-purpurin (3), pongamol (4), lanceolatin B (5), (-)-maackiain (6), (-)-3-hydroxy-4-methoxy-8,9-methylene-dioxypterocarpan (7), and (-)-medicarpin (8), were obtained as active compounds from *Tephrosia purpurea*[9].

Since Diabetes is a chronic metabolic disorder, has been treated with several medicinal plants or their extracts based on folklore medicine.[10] Synthetic hypoglycaemic agents can produce serious side effects and in addition, they are not suitable for use during preganacy. Therefore, the search for more effective and safer hypoglycaemic agents has continued to be an important area of active research.[11] Furthermore, after the recommendations made by WHO on diabetes mellitus, investigations on hypoglycaemic agents from medicinal plants have become more important.[12]

Many herbal medicines have been recommended for the treatement of diabetes. Traditionally plant medicines are used throughout the world for a range of diabetic conditions.

Materials and Methods

Drugs and chemicals

Alloxan hydrate (Spectrochem Pvt. Ltd. Mumbai), Glibencamide (Aventis Pharma Ltd., Mumbai, India) and Glucose Kit, Enzymatic GOD- POD method (Span Diagnostics Ltd., Sachin Surat) were used. All the solvents used for the extraction process are of laboratory grade obtained from S.D. fine chemicals, Mumbai.

Plant extraction[13]

The roots of the plant were collected in the month of April – June 2007 and authentified by Dr.K.P.Sreenath, Reader and Taxonomist, Botany Department from Bangalore University. A sample specimen was deposited, bearing voucher number **PESNCJ-07.** The shade dried plant material was powdered. The coarse powder was subjected to successive extraction with Pet-Ether 60-80^0, alcohol and aqueous alcohol (60% water + 40% ethanol) by maceration. Each extract was concentrated under vacuum to give Petroleum ether extract [PE] (1.28%w/w), alcoholic extract [AE] (6.82%w/w) and hydro-alcoholic extract [HAE] (1.14%w/w).

Phytochemical investigation

The various extracts of *Tephrosia purpurea* was subjected to preliminary qualitative investigations [14]

Experimental animals

Wistar albino rats of either sex weighing 150–200 g were used for hypoglycemic and antidiabetic studies. They were housed in standard environmental conditions [temperature 22 ± 1^{0}C; relative

humidity 55-60% and 12:12 h light/dark cycle) and fed with standard rodent diet with water *ad libitum*. All animal procedures were carried out after obtaining clearance from Institutional Animal Ethical Committee. Eight groups of six animals were used for each experiment.

Preparation of drugs

The extracts of Pet Ether 60-80^0 and Alcohol were insoluble in water and aqueous alcohol extract of *Tephrosia purpurea* were freely soluble in water. All the extracts were suspended in 1% Tween-80 and used for oral administration. Each time fresh preparations of the extracts were prepared when required.

Hypoglycaemic studies on normal rats[15]

Fasted rats were divided in to 8 groups consisting of 8 animals in each group. First group received vehicle (3% v/v tween 80 in distilled water, orally in a volume of 10 ml/kg, which served as control). Group II received Glibenclamide (5 mg/kg, p.o.) as standard drug suspended in vehicle. The other six groups received various extracts at two different dose levels for a period of 7 days. Blood samples were collected from the tail vein or by retro-orbital puncture method just prior to and at 1, 2, 4 h after dosing for acute studies and glucose was estimated. For sub-acute studies blood sample was removed on 4th and 7th day after 16 h of overnight fasting for glucose level estimation.

Oral glucose tolerance test (OGTT) in normal rats[15]

Oral glucose tolerance test (OGTT) was carried by administering glucose (2 g/kg, p.o.), 30 minutes after the extract or standard drug administration after 7 days of pretreatment period. Blood samples were collected from tail vein for glucose analysis prior to glucose administration (0 h) and at 1, 2 and 3 h after glucose loading.

Induction of diabetes

Diabetes was induced in rats by single injection of alloxan monohydrate (120 mg/kg, i.v.) in ice cold normal saline. The rats after Alloxan injection were given 5% w/v glucose solution in feeding bottles for next 24 hours in their cages to prevent hypoglycemia. Five days after the injection of alloxan, the animals were fasted for 16 h and blood sugar was determined to the surviving animals. Rats with blood glucose level >200 mg/dl were considered diabetic and were used in the experiment.

Antidiabetic studies of extracts on alloxan induced diabetic rats

Diabetic rats were divided into 8 groups consisting of 8 animals in each group. Group I received vehicle (3 %v/v tween 80, p.o.) which served as control. Group II received glibencamide as reference drug (5 mg/kg, p.o.) suspended in vehicle. The other six groups received various extracts at two different dose levels for a period of 7 days. Blood samples were collected from the tail vein just prior to and at 1, 2 and 4 hour after single dosing. In subacute studies weekly blood samples were collected by retro-orbital puncture method after 1 h of extract administration on 7th day. OGTT was performed at the end of 7 day study period. Blood glucose was measured. Weekly body weight and mortality were also recorded at the end of study period

Determination of blood glucose concentration

Blood glucose concentration was determined in plasma by commercially available glucose kit based on glucose oxidase method. Instruction manual provided by manufacturer was followed to obtain the glucose level present in the sample and was expressed as mg/dl.

Statistical analysis

Values are expressed as mean ± SEM from 8 animals. Statistical differences in mean were analyzed using one way ANOVA (analysis of variance) followed by Dunnett's test. $p<0.05$ was considered significant.

Results

Effect of extracts of** Tephrosia purpurea **on blood glucose concentration in normal rats

Single dose administration of PE, AE and HAE (100 and 200 mg/kg) did not significantly reduce the fasting blood glucose level {FBG) at various time intervals viz. 1, 2 and 4 h after treatment, indicating that the extracts could not produce significant hypoglycaemic activity after acute treatment (Table 1). Repeated administration of AE and HAE (100 and 200 mg/kg) for 7 days significantly reduced the FBG (Table 1). However PE did not possess any hypoglycaemic effect even after treatment for 7 days in normal rats. Glibenclamide (5 mg/kg) significantly reduced blood glucose level (BGL) after single dose and repeated dose administration as compare to vehicle groups (Table 1).

Table 1 Effect of *extracts of Tephrosia purpurea* on blood glucose concentration in normal rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		On day 1				On day 7 (1 h after drug administration)
		0 h	1 h	2 h	4 h	
Vehicle, 5% Tween 80	10 ml/kg	98.83 ±2.23	96.89 ±2.22	95.73 ±1.84	96.50 ±1.44	96.5 ±0.42
Glibenclamide	5	96.89±1.29	84.88 ±0.52**	74.8 ±0.71**	64.73±0.71**	64.73 ±0.70**
PE	100	100.50±0.81	101.01 ±1.10	99.67 ±1.36	100.5 ±1.5	95.17 ±1.49
PE	200	100.67 ±1.08	100.00 ±1.04	99.66 ±0.92	100.17 ±0.75	97.33 ±0.92
AE	100	95.83 ±2.12	97.04 ±1.36	95.83 ±1.82	95.33 ±2.30	85.17 ±1.04**
AE	200	99.01 ±1.71	95.67 ±2.13	97.67 ±1.26	96.5 ±1.26	71.83 ±1.62**
HAE	100	100.50 ±0.80	100.33 ±0.88	100.83 ±0.81	99.67 ±1.358	90.83 ±1.78*
HAE	200	102.67 ±1.14	101.34 ±1.36	99.5 ±0.67	101.33 ±1.36	90.5 ±1.36*

Values are expressed as mean ± S.E.M from 8 rats. *$P<0.05$; **$P<0.01$ significant from the control animals.

Administration of glucose (2g/ kg) to 7 days pretreated rats significantly suppressed the rise in BGL with glibenclamide (5 mg/kg) AE and HAE (100 and 200 mg/kg) at 1, 2 and 3 h as compare with vehicle control. PE did not produce any hypoglycaemic effect in glucose loaded rats (Table 2). Out of various extracts AE possessed maximum activity.

Table 2 Effect of *extracts of Tephrosia purpurea* of blood glucose level of glucose loaded hyperglycaemic rats (OGTT) after 7 days treatment.

Treatment	Dose (mg/kg)	Percentage change in blood glucose level (mg/dl)		
		1 h	2 h	3h
Vehicle, 5% Tween 80	10 ml/kg	51.77 ±3.18	32.55 ±1.31	12.42 ±1.66
Glibenclamide	5	28.24** ±0.47	10.89** ±0.46	1.01** ±0.45
PE	100	50.49 ±0.17	29.59 ±0.64	11.51 ±0.66
PE	200	47.95 ±1.03	28.83 ±0.14	12.21 ±0.24
AE	100	32.93 **±0.19	14.04** ±0.53	3.46** ±0.5
AE	200	30.87 **±0.52	12.92** ±0.51	2.88 **±0.62
HAE	100	43.17 ±0.66	21.16* ±0.58	8.89 ±0.52
HAE	200	42.01* ±1.32	19.32 **±0.75	6.94*±0.49

Values are expressed as mean ± S.E.M from 8 rats. *P<0.05; **P<0.01 significant from the control animals.

Effect of extracts of** Tephrosia purpurea **on blood glucose concentration in diabetic rats

Treatment with alloxan (120 mg/kg, i.v.) had increased the blood glucose level (BGL) to a range of 250-270 mg/dl after 5 days. Single dose administration of PE, AE and HAE (100 and 200 mg/kg) did not significantly reduced the BGL in alloxan induced diabetic rats, while glibenclamide (5 mg/kg) significantly reduced the BGL at 1st, 2nd and 4th hour after single dose administration in alloxan induced diabetic rats (Table 3).

Table 3 Effect of *extracts of Tephrosia purpurea* on blood glucose concentration in alloxan induced diabetic rats.

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)				
		On day 1				On day 7 (1 h after drug administration)
		0 h	1 h	2 h	4 h	
Diabetic control (Vehicle)	10 ml/kg	252.77 ±9.27	252.45 ±9.20	251 ±9.06	248.96 ±8.81	256.06 ±7.76
Glibenclamide	5	252.38 ±9.07*	214.15 ±3.19**	183.57±1.36**	154.76±4.88**	99.62 ±0.93**
PE	100	246.82 ±9.49	242.45 ±8.50	233.73 ±8.83	223.42 ±6.308	245.83 ±1.53
PE	200	247.22 ± 9.18	244.04 ±8.28	234.52 ±7.42	227.77 ±6.94	244.16 ±1.53
AE	100	238.89 ±10.05	235.31 ±10.60	226.98 ±9.69	219.04 ±10.17	133.16±2.34**
AE	200	247.61 ±9.20	241.66 ±8.21	236.9 ±8.07	228.57 ±8.64	125.43±1.16**
HAE	100	253.97 ±10.76	245.64 ±11.36	232.15 ±9.25	224.59 ±9.35	215.83 ±2.71*
HAE	200	245.24 ±7.99	239.28 ±7.89	230.52 ±8.62	228.318 ±8.30	214.17 ±3.74*

Values are expressed as mean ± S.E.M from 8 rats. *P<0.05; **P<0.01 significant from the control animals.

Repeated dose administration with AE and HAE (100 and 200 mg/kg) significantly reduced the BGL in a dose dependent manner over a period of 7 days (Table 3) and improved the glucose tolerance (Table 4). These results indicate the AE and HAE possessed hypoglycaemic activity on repeated administration in alloxan induced diabetic rats. However there is no marked change in glucose levels were observed with PE even after repeated dose administration. It was observed that AE possessed greater hypoglycaemic activity in alloxan induced diabetic rats.

Table 4 Effect of *extracts of Tephrosia purpurea* of blood glucose level of glucose loaded hyperglycaemic rats (OGTT) after 7 days treatment

Treatment	**Dose (mg/kg)**	**Percentage change in blood glucose level (mg/dl)**		
		1 h	**2 h**	**3h**
Diabetic control (Vehicle)	10 ml/kg	53.15 ±1.4	30.94 ±0.4	13.71 ±0.56
Glibenclamide	5	27.51** ±0.54	9.15** ±1.56	1.75** ±0.73
PE	100	50.98 ±1.55	29.41 ±1.77	13.73 ±0.72
PE	200	47.86 ±0.68	28.40 ±0.62	10.89 ±1.8
AE	100	33.24 **±1.26	14.59 **±0.68	4.59** ±0.25
AE	200	29.76 **±0.57	10.98 **±0.59	2.76** ±0.68
HAE	100	43.93* ±0.29	21.66 *±1.76	7.84 ±0.89
HAE	200	42.63* ±2.8	20.12* ±0.92	6.40* ±0.31

Values are expressed as mean ± S.E.M from 8 rats. *P<0.05; **P<0.01 significant from the control animals.

Discussion

From the present study it is clear that PE, AE and HAE did not possess any hypoglycaemic activity in normal rats after single dose administration. On the other hand 7 days pretreatment with AE and HAE (100 and 200 mg/kg) significantly reduced the blood sugar levels. These studies indicated that PE may not any hypoglycaemic or antidiabetic activity. Further treatment of 7 days is essential for the extracts to show its therapeutic activity. Hence for the alloxan induced diabetic study extracts were treated for 7 days and the hypoglycaemic activity was assessed.

Administration of alloxan (120 mg/kg, i.v.) produced significant stable increase in BGL after 5 days. Alloxan chemically 2,4,5,6-tetraoxo pyrimidine is a potent destructor of pancreatic β-cells through generation of reactive oxygen species (ROS). It has been postulated that glucose transporter and glucokinase are the target molecule for alloxan, leading to decreased insulin levels and uncontrolled BGL. The hyperglycemia seen in alloxan induced diabetic rats in our study may be due to the above mentioned mechanism.

Single dose administration of PE, AE and HAE could not control BGL in alloxan induced diabetic rats. However, repeated dose administration of AE and HAE significantly controlled the elevated BGL on 7th day in alloxan induced diabetic rats compared to diabetic treated group.

Repeated administration of AE and HAE improved the glucose tolerance in alloxan induced diabetic rats on 7th day as compared to diabetic control. Alloxan at high dose causes irreversible damage of β-cells leading to Type-I diabetes. Lack of insulin in alloxan induced diabetic rats, leads to impaired glucose tolerance. From the present study, it was demonstrated that, AE and HAE could improve the glucose tolerance, suggesting that these extracts may show insulinomimetic activity or improved glucose utilization mechanism.

From these studies we can conclude that alcoholic and hydro-alcoholic extract of *Tephrosia purpurea* could possess hypoglycaemic activity in normal and alloxan diabetic rats. Petroleum ether extract did not possess any hypoglycaemic activity. Among the various extracts tested alcoholic extract had greater activity compared to hydro-alcoholic extract. Further studies are required to isolate the active constituent and the mechanism of hypoglycemia produced by the active constituents.

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