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ARTICLE *in* JOURNAL OF ETHNOPHARMACOLOGY · MAY 2009

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Journal of Ethnopharmacology 124 (2009) 111–115

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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Hypoglycemic and antihyperglycemic effect of *Begonia malabarica* Lam. in normal and streptozotocin induced diabetic rats

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ARTICLE INFO

Article history:
Received 26 December 2008
Received in revised form 24 February 2009
Accepted 1 April 2009
Available online 10 April 2009

Keywords:
Begonia malabarica Lam. (Begoniaceae)
Streptozotocin
Diabetes

ABSTRACT

Aim of the study: The stem of *Begonia malabarica* was used traditionally by the *Malasar* tribe to treat diabetes. To validate the hypoglycemic and antihyperglycemic effects of the hexane, ethylacetate and methanol extracts obtained from an ethnomedicinal plant, *Begonia malabarica*.
Materials and methods: The doses for the study were fixed based on Irwin test. The hypoglycemic effect of hexane, ethylacetate and methanol extracts of *Begonia malabarica* stems were studied in normal animals. The antihyperglycemic effect of the methanol extract was studied in streptozotocin induced diabetic rats.
Results: In normal rats the treatment with the methanol extract of *Begonia malabarica* had shown a highly significant reduction (16.54 and 34.47%) in plasma glucose levels from the 0 h values at the dose of 100 and 200 mg/kg respectively. In streptozotocin induced diabetic rats the body weight of the *Begonia malabarica* methanol extract treated animals had shown a significant increase (13.38% at 200 mg/kg) after 4 weeks treatment. The plasma glucose levels were reduced significantly by 46.57 and 50.20% after 4 weeks treatment at 100 and 200 mg/kg respectively. Likewise the absolute kidney weight was also reduced in a significant manner. After 25 days treatment the *Begonia malabarica* methanol extract treated animals had shown low fasting plasma glucose levels (54.29, 61.34% in 100 and 200 mg/kg) and reduced postprandial plasma glucose levels (54.23, 65.96% in 100 and 200 mg/kg) when compared with diabetic control values. Serum insulin levels and liver glycogen levels were increased to 40.04 and 42.18% in 200 mg/kg *Begonia malabarica* methanol extract treated animals respectively. The treatment with *Begonia malabarica* methanol extract did not change the triglycerides and total cholesterol levels. The urea and creatinine levels were also reduced significantly by this treatment. The reduction in SGPT levels indicated the absence of toxicity of *Begonia malabarica* extract at this dose level.
Conclusion: This study supports the use of *Begonia malabarica* by the *Malasar* tribe for the treatment of diabetes. Fractionation of this extract may yield novel prototypes to manage diabetes mellitus.
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1. Introduction

Diabetes is a common endocrine disease characterized by the metabolic abnormalities and by the long term complications involving eyes, kidneys, nerves and blood vessels. It is considered a "modern day epidemic" and is rightly recognized as a global public health issue. The number of adults with diabetes in the world will rise to 300 million by the year 2025 and the major part of this numerical increase will occur in developing countries (King et al., 1998). Trends in the last 10 years are influencing the supply and demand for healthcare in diabetes (Bottomely and Raymond, 2007).

The UK perspective diabetic study (UKPDS) had indicated that the intensive glycemic control reduced the disease complications and mortality (Adler et al., 2000; Stratton et al., 2000). Many Indian plants have been reported by various authors to treat diabetes traditionally (Mukherjee et al., 2006).

An ethnomedical study carried out by Entomology Research Institute, Chennai (India) showed that the stems of *Begonia malabarica* Lam. (Begoniaceae) was used as a remedy for diabetes by the *Malasar* tribe, in Coimbatore District (Pandikumar et al., 2007). *Begonia malabarica* is a subshrub occurring in occasional clumps above 900 MSL, in peninsular India and Sri Lanka. The stem is succulent, reddish and the leaves are inequilateral. The flowers are unisexual and the number of tepals is two. The fruit is a capsule (Matthew, 1991). Friedelin, epi-friedelinol, β-sitosterol, luteolin, quercetin and β-sitosterol-3-β-D-glucopyranoside were reported from the leaves of *Begonia malabarica*. Begonanline, nantoamide, and methyl-(*S*)-glycerate were isolated from *Begonia nantoensis*. Cucurbitacin B, E, I and dihydrocucurbitacin isolated from *Begonia nantoensis* were reported to have cytotoxicity in cancer cell lines. 22a-Dihydroxyolean-12-en-29-oic acid, indole-3-

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0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.04.001

carboxylic acid, 5,7-dihydroxychromone, and (2)-catechin isolated from *Begonia nantoensis*, significantly reduced HIV replication in H9 lymphocyte cells (Wu et al., 2004). The methanol and chloroform extracts of *Begonia malabarica* were shown to have antibacterial activity against *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio parahaemolyticus*, and *Chromobacterium violaceum* (Ramesh et al., 2002). The present study was undertaken to assess the hypoglycemic and antihyperglycemic activity of the extracts of *Begonia malabarica*.

2. Materials and methods

2.1. Plant material

Stems of *Begonia malabarica* were collected from Poondi, Coimbatore District, Tamil Nadu, in November 2007. The botanical identity of the plant material was confirmed by the Taxonomist at Department of Botany, Loyola College, Chennai. A voucher specimen (ERIP-4) was deposited in the herbarium of Entomology Research Institute.

2.2. Preparation of the extracts

The fresh plant materials were washed thoroughly with water and crushed. Two kilograms of fresh plant material was extracted with 6 l of methanol three times by cold percolation method. The combined extracts were concentrated under reduced pressure to give brick red syrup. Then the extract was suspended in water (1:1) and partitioned with hexane and ethylacetate successively. The percent yield for hexane, ethylacetate and methanol extracts was 0.271, 0.889 and 5.204% (w/w) respectively.

2.3. Preliminary phytochemical screening of the active extract

The preliminary phytochemical tests were carried out for the effective extracts using standard phytochemical methods (Harborne, 1998).

2.4. Animals

Male albino rats (Wistar strain) weighing 140–170 g were kept in polypropylene cages, under controlled temperature, humidity and 12/12 h light/dark cycles. The animals were fed *ad libitum* with normal laboratory chow diet containing 74% carbohydrate, 22% protein and 4% fat, purchased from Pranav Agro Industries Ltd., Maharashtra. This study got clearance from the Institutional Animal Ethical Committee (IAEC-ERI-LC-11).

2.5. Fixation of doses

The doses for the study were fixed based on Irwin test for the extracts at 1, 2, 3, 4 and 5 g/kg (Roux et al., 2004). The extracts were dissolved in a vehicle containing 0.2% polysorbate–80, 0.5% sodium carboxy methyl cellulose, 0.9% sodium chloride, 0.9% benzyl alcohol and 97.2% distilled water (Lee, 2001). Non-diabetic, male rats weighing 150 ± 5 g were used in this study. Three animals were used for each group. On the day preceding the experiment the animals were appropriately grouped and placed in the experiment room for acclimatization. On the morning of the experiment day, food and water were removed from the cages. Then the animals were treated orally with the vehicle or the extracts. At 0, 15, 30, 60, 120, 180 min and 24 h after treatment of the extracts behavioral alterations were observed. 1/10th–1/20th of the dose in which behavioral alterations were observed, was considered safe dose for further assays (Oliveira et al., 2008).

2.6. Assessment of hypoglycemic effect of the extracts in normal rats

The hypoglycemic effect of the extracts was studied at 50, 100 and 200 mg/kg. The animals were fasted for 12 h. At 0 h, blood samples were collected from the retro orbital sinus. Then the animals were treated with the extracts dissolved in vehicle. The normal control animals were treated with the vehicle alone. Glibenclamide (5 mg/kg) was used as positive control. All the treatments were given orally. At 1, 2 and 4 h after the treatment with the extracts, blood samples were collected for plasma glucose estimation.

2.7. Assessment of antidiabetic effect of the methanol extract of *Begonia malabarica* in streptozotocin induced diabetic rats

2.7.1. Induction of diabetes

Six-week old, male albino rats weighing 145 ± 5 g were used in the study. Diabetes was induced according to the procedure of Wu and Huan (2008) but the amount of streptozotocin used to induce diabetes was 40 mg/kg. 40 mg/kg of streptozotocin in acidified saline with 50 mM sodium citrate buffer (pH 4.5) was injected intraperitonealy. On 10th day after the induction blood samples were collected for the estimation of plasma glucose levels. Animals having plasma glucose levels ≥200 mg/dL were included in the study.

2.7.2. Treatment with extracts

Based on the body weight and plasma glucose levels the animals were assigned randomly in to five groups with six animals in each group. Group I was normal rats treated with vehicle, Group II was diabetic rats treated with vehicle, Group III was diabetic rats which received glibenclamide (5 mg/kg) (Habibuddin et al., 2008), Group IV was diabetic rats which received *Begonia malabarica* methanol extract (100 mg/kg), and Group V was diabetic rats which received *Begonia malabarica* methanol extract (200 mg/kg). Treatments were given orally between 12.00 p.m. and 2.00 p.m., once daily, continuously for 4 weeks.

2.7.3. End of the study

At the end of the study the animals were sacrificed between 9.00 a.m. and 11.00 a.m. to minimize the diurnal variations. The animals were euthanized and blood was collected on EDTA containing tubes. Liver samples were sliced quickly, snap-freezed in liquid N_2, stored at −70 °C for the analysis of glycogen.

2.7.4. Biochemical analysis

Food and water intake was measured daily between 6.00 p.m. and 7.00 p.m. Plasma glucose levels and body weight were measured weekly between 9.00 a.m. and 11.00 a.m. At the 25th day after treatment OGTT was performed. Briefly, the animals were fasted from 7.00 a.m. to 1.00 p.m. and 0 h samples were taken. Then the extracts were administered to the animals. Thirty minutes after the treatment with extracts 2 g/kg glucose solution was given to the animals. At 30 and 120 min after the glucose load blood samples were drawn from the animals for glucose estimation (Matteucci and Giampietro, 2008). All other parameters were estimated at the end of the study. Plasma glucose levels were measured by GOD–POD method. Serum insulin levels were measured by microplate ELISA method using a commercial kit. Total cholesterol levels were measured by CHOD–POD method. Triglyceride levels were estimated by GPO–POD method. Urea nitrogen levels were measured by the modified DAM method (Wybenga et al., 1971). Creatinine levels were measured by the modified Jaffe's kinetic method. SGOT and SGPT levels were measured by spectrophotometric method which involves NADH oxidation. Liver glycogen levels were measured by the method of Carroll et al. (1956). Kidneys and retroperitoneal

Table 1
Hypoglycemic effect of *Begonia malabarica* extracts in normal animals at 50, 100 and 200 mg/kg.

Groups	Zero hour	First hour	Second hour	Fourth hour
Normal control	90.14 ± 2.06	88.48 ± 2.66	86.48 ± 0.78	90.22 ± 1.97
Hexane extract (50 mg/kg)	88.50 ± 3.55	81.30 ± 3.25	83.57 ± 4.88	81.05 ± 3.50
Hexane extract (100 mg/kg)	89.48 ± 2.49	85.04 ± 4.63	87.46 ± 1.27	85.91 ± 3.86
Hexane extract (200 mg/kg)	88.35 ± 1.75	87.39 ± 3.42	87.05 ± 2.92	87.14 ± 2.81
Ethylacetate extract (50 mg/kg)	88.55 ± 3.04	86.04 ± 2.15	87.80 ± 2.51	88.43 ± 3.29
Ethylacetate extract (100 mg/kg)	88.34 ± 3.34	89.89 ± 2.33	92.90 ± 1.79	92.87 ± 1.90
Ethylacetate extract (200 mg/kg)	92.92 ± 2.86	97.84 ± 3.35	90.90 ± 2.91	104.54 ± 5.98
Methanol extract (50 mg/kg)	90.49 ± 2.11	87.23 ± 3.85	83.55 ± 1.27*	79.59 ± 4.72
Methanol extract (100 mg/kg)	90.77 ± 2.23	78.50 ± 3.04*	82.93 ± 2.25*	75.75 ± 2.81**
Methanol extract (200 mg/kg)	89.85 ± 2.42	73.36 ± 3.10*	67.13 ± 2.68**	58.87 ± 1.99**
Glibenclamide (5 mg/kg)	85.57 ± 7.69	41.98 ± 4.97*	44.23 ± 4.03**	42.88 ± 5.29**

All values are (in mg/dL) mean ± SEM for four animals.
* Values deviate significantly from corresponding 0 h values ($P \leq 0.05$).
** Values deviate very significantly from corresponding 0 h values ($P \leq 0.005$).

fat pads were removed, weighed and expressed as per cent body weight.

2.8. Statistical analysis

One-way ANOVA and Student's *t*-test (SPSS Program; Version 12.0) were used to compare the data with the level of significance set at $P \leq 0.05$.

3. Results

The methanol extract of *Begonia malabarica* gave positive results for Lieberman Burchard test, Nollers test and anthrone test. This indicated the presence of steroids, terpenoids, and carbohydrates in the extract. It formed a purple colour when treated with 1% methanolic hydrochloric acid indicating the presence of anthocyanins. Further it gave positive results for tannins, carboxylic acids and saponins. Alkaloids were absent in this extract.

Within 10–15 min after the treatment of the extracts at 4 and 5 g/kg, the animals had shown ptosis, loss of balance, rolling gait, lacrimation, salivation and abdominal writhes. There was no mortality in the animals treated with the extracts at the dose of 4 and 5 g/kg. No adverse effects were found in the animals treated with 1, 2 and 3 g/kg of extracts. Therefore the dose range for the extracts was fixed between 50 and 200 mg/kg, for further studies.

In normal animals the treatment with methanol extract of *Begonia malabarica* at 50 mg/kg reduced the plasma glucose levels by 7.66 and 12.04% at third and fourth hours after treatment, respectively. At 100 mg/kg there was a highly significant reduction in plasma glucose levels by 8.63 and 16.54% at third and fourth hour after the treatment of the extract. A highly significant reduction (25.28 and 34.47%) in plasma glucose levels was found in 200 mg/kg extract treated animals. The hexane and ethylacetate extracts treated animals failed to show any significant reduction in plasma glucose levels. The glibenclamide treated animals had shown 48.31 and 49.88% reduction in plasma glucose levels when compared with 0 h values, respectively (Table 1).

In streptozotocin induced diabetic animals the treatment with *Begonia malabarica* methanol extract at 200 mg/kg significantly increased the body weight (13.38%) (Fig. 1) reduced feed and water consumption, reduced absolute kidney weight (Table 2) and plasma glucose levels (Fig. 2). In *Begonia malabarica* methanol extract treated animals at a dose of 100 mg/kg there was no significant increase in body weight. The plasma glucose levels were reduced in a significant manner. Likewise, the feed and water intake, absolute kidney weight were also reduced significantly. In oral glucose tolerance test, the treatment with the extracts had reduced the fasting as well as the postprandial plasma glucose levels significantly (Table 3). The serum insulin levels and liver glycogen levels were increased significantly. The total cholesterol and triglyceride levels were not affected by the treatment with *Begonia malabarica* methanol extract at 100 and 200 mg/kg. The urea, creatinine and SGPT levels were reduced by the treatment with the extract (Table 4).

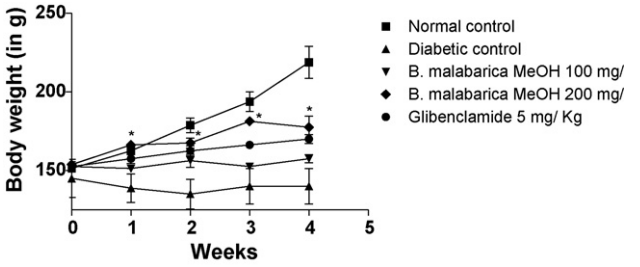

Fig. 1. Effect of *Begonia malabarica* methanol extract on body weight of STZ diabetic rats after 4 weeks treatment.

4. Discussion

The present study reports for the first time the effect of *Begonia malabarica* as a hypoglycemic and antihyperglycemic agent, thus scientifically validating the traditional claim. The methanolic extract of *Begonia malabarica* had shown a significant hypoglycemic effect in normal rats at third and fourth hour after treatment. The mechanism of action of hypoglycemic activity of the extract was not studied; however it has already been reported that this hypoglycemic action might be due to modulation of insulin secretion and/or insulin action or could be related to the interference on absorption of dietary carbohydrates as well as disaccharides in small intestine leading to the suppression of meal induced increase of plasma glucose (Ortiz-Andrade et al., 2007).

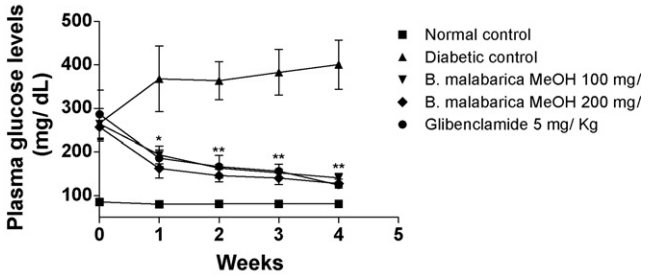

Fig. 2. Effect of *Begonia malabarica* methanol extract on plasma glucose level of STZ diabetic rats after 4 weeks treatment.

Table 2
Effect of *Begonia malabarica* methanol extract on feed intake, water intake, and organ weights of STZ diabetic rats after 4 weeks treatment.

Groups	Feed intake (g/day)	Water intake (mL/day)	Weight of the kidney (mg/100 g BW)	Weight of retroperitoneal fat (mg/100 g BW)
Normal control	18.21 ± 0.25	23.03 ± 0.25	312.66 ± 12.05	961.77 ± 67.92
Diabetic control	38.03 ± 0.37	100.89 ± 1.21	444.17 ± 32.87	314.15 ± 110.82
Begonia malabarica methanol extract (100 mg/kg)	21.07 ± 0.32**	63.57 ± 0.50**	338.19 ± 12.62*	594.90 ± 134.51
Begonia malabarica methanol extract (200 mg/kg)	19.82 ± 0.57**	49.10 ± 1.51**	377.59 ± 06.83*	480.13 ± 25.57
Glibenclamide (5 mg/kg)	19.79 ± 0.99**	36.66 ± 0.52**	353.64 ± 09.45*	898.41 ± 44.46**

All values are (in g) mean ± SEM.
* Values deviate significantly from diabetic control group ($P \leq 0.05$).
** Values deviate very significantly from diabetic control group ($P \leq 0.005$).

Table 3
Effect of *Begonia malabarica* methanol extract on OGTT pattern of STZ diabetic rats after 25 days treatment.

Groups	Plasma glucose levels (mg/dL)		
	Zero minutes	Thirty minutes	One twenty minutes
Normal control	89.52 ± 08.36	136.83 ± 08.53	113.11 ± 11.31
Diabetic control	286.47 ± 49.19	344.54 ± 39.77	302.70 ± 49.91
Begonia malabarica methanol extract (100 mg/kg)	130.92 ± 05.47*	158.90 ± 03.02*	138.54 ± 06.86**
Begonia malabarica methanol extract (200 mg/kg)	110.73 ± 02.52*	131.85 ± 08.35**	103.02 ± 04.16**
Glibenclamide (5 mg/kg)	94.72 ± 05.83**	143.97 ± 10.36**	78.91 ± 08.88**

All values are (in mg/dL) mean ± SEM for six animals.
* Values deviate significantly from diabetic control group ($P \leq 0.05$).
** Values deviate very significantly from diabetic control group ($P \leq 0.005$).

In the present study, streptozotocin produced significant increase in plasma glucose level by 260–300 mg/dL by selectively destroying the pancreatic insulin secreting β-cells causing diabetes close to type 2 diabetes of humans. After 25 days treatment, 100 mg and 200 mg/kg, methanol extract of *Begonia malabarica* had reduced both the fasting as well as postprandial plasma glucose levels in streptozotocin induced diabetic rats. Our results show that decreased postprandial glucose in animals may be correlated with decreased gluconeogenic activity with reduced urea excretion or inhibition of glycogenolysis as suggested by increased liver glycogen (Oliveira et al., 2008). OGTT was studied for streptozotocin induced rats at the 25th day after treatment. Both 100 and 200 mg/kg methanol extract showed a significant reduction in plasma glucose level. Likewise, the insulin levels also increased suggesting the possible action of improved tissue glucose uptake. We had studied the alpha glucosidase inhibitory effect of the methanol extract of *Begonia malabarica*. The extract did not exhibit any significant alpha glucosidase inhibition up to 500 μg/mL concentration (data not shown). These results suggest that the efficacy of the extract may be due to increase in the insulin secretion and not due to the reduction in digestion or absorption of carbohydrates.

In methanol extract treated diabetic rats the reduction in urea level indicated reduced proteolysis (Oliveira et al., 2008) and this might be the reason for the increase in the body weight of the animals. The entry of renal glucose is not dependent on action of insulin and therefore in the event of hyperglycemia there is an increase in the entry of glucose (Belfiore et al., 1986). This has been postulated to cause increased intra-renal glycogen deposition which leads to glycosylation of basement membrane collagen in kidney (Anderson and Stowring, 1973). Therefore the weight of kidneys increases in diabetic condition (Raju et al., 2001). The reduction of the kidney weight in the extract treated animals indicates that the treatment could prevent the onset of macrovascular complications. Likewise, the reduction in urea and creatinine levels indicates the renoprotective effect of the extract.

The treatment with this extract did not affect the total cholesterol and triglyceride levels. This suggests that the mode of action of this extract does not affect lipid metabolism.

At the doses of 4 and 5 g/kg, the extract showed some effect on autonomic functions and CNS depression. But the animals had recovered within 5 h after treatment and there was no mortality. No toxic signs were observed below 4 g/kg. Further in streptozotocin induced animals, at the dose levels of 100 and 200 mg/kg, the reduction in urea, creatinine and SGPT levels indicated the absence of any toxic effects (Table 4).

In conclusion, our study adds credence to the traditional use of *Begonia malabarica* by the *Malasar* tribe to treat diabetes.

Table 4
Effect of *Begonia malabarica* methanol extract on lipid profile and other biochemical parameters of STZ diabetic rats after 4 weeks treatment.

Groups	Insulin (μU/mL)	Liver glycogen (mg/g liver)	Total cholesterol (mg/dL)	Triglycerides (mg/dL)	Urea nitrogen (mg/dL)	Creatinine (mg/dL)	SGOT (U/L)	SGPT (U/L)
Normal control	16.24 ± 0.19	13.73 ± 0.32	62.05 ± 02.40	44.16 ± 02.58	12.12 ± 0.87	0.30 ± 0.01	138.00 ± 2.48	58.75 ± 4.53
Diabetic control	7.29 ± 0.29	7.03 ± 0.39	80.57 ± 03.42	136.36 ± 06.69	33.92 ± 6.07	1.08 ± 0.08	157.75 ± 28.23	122.00 ± 8.53
Begonia malabarica methanol extract (100 mg/kg)	10.79 ± 0.22**	10.12 ± 0.25**	89.58 ± 02.08	141.17 ± 02.47	15.74 ± 0.52*	0.40 ± 0.08**	110.12 ± 4.44	77.75 ± 5.46**
Begonia malabarica methanol extract (200 mg/kg)	12.16 ± 0.29**	12.16 ± 0.22**	87.49 ± 2.40	139.07 ± 6.48	16.94 ± 0.80*	0.41 ± 0.08**	121.00 ± 7.31	85.75 ± 2.86**
Glibenclamide (5 mg/kg)	12.98 ± 0.24**	12.53 ± 0.37**	69.57 ± 03.23*	84.09 ± 12.82*	13.67 ± 0.95*	0.30 ± 0.01**	129.25 ± 6.40	73.00 ± 5.58*

All values are (in mg/dL) mean ± SEM for six animals.
* Values deviate significantly from diabetic control group ($P \leq 0.05$).
** Values deviate very significantly from diabetic control group ($P \leq 0.005$).

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