

Evaluation of Antidiabetic activity of methanolic root extract of *Caesalpinia digyna* (Rottler) on rat

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Abstract

The methanolic root extract of *Caesalpinia digyna* Rottler (MECD) was tested for its antidiabetic activity using normoglycaemic plus glucose fed hyperglycaemic rats (NG-OGTT) and by acute treatment method in streptozotocin-induced diabetic rats. Blood glucose levels were determined after oral administration of two concentrations of MECD (250 and 500 mg/kg) at specific time intervals. Glibenclamide (0.4mg/kg) was taken as a standard reference. The results revealed that MECD at a dose level of 500mg/kg exhibit significant ($p < 0.01$) hypoglycaemic effect in NG-OGTT model compared to low dose level of 250mg/kg ($p < 0.05$). In streptozotocin-induced diabetic rats both the concentrations of MECD showed significant ($p < 0.01$) hypoglycaemic effect in acute treated groups. As both concentrations of MECD shows significant hypoglycaemic effect in the two models it was further recommended to evaluate MECD for its long standing antidiabetic effect and the molecular mechanism for its antidiabetic activity thereby justifying the use of the plant in the indigenous system of medicine.

Key words: *Caesalpinia digyna* root; NG-OGTT, Streptozotocin, Hypoglycaemic, Glibenclamide

Introduction

Caesalpinia digyna Rottler (Family: Leguminosae) is a large, scandent, prickly shrub or climber, up to 10m in height, growing wild in the scrub forests of the eastern Himalayas in Assam and West Bengal, the Eastern Ghats in Andhra Pradesh, Madhya Pradesh and also in Ceylon and Malay Islands. The pods popularly known as Teri pods, contains about 28 percent of tannin, where as pods with seeds contain more than 54 percent tannin. The bark also contains tannin of 28 percent. The tannin is a pure gallo-tannin and gallic acid (Anon, 1992) Chemical investigations of the plant have shown the presence of caesalpinine A, cellalocinnine, ellagic acid, Gallic acid, bergenin, bonducellin, intricatinol and tannins.

The plant is one of the ingredients of an indigenous drug preparation 'Geriforte', which has been used for curing senile prurites with excellent results. Root has marked astringent properties and intoxicating effect. It exhibit antifatigue and antioxidant effect in rats. The ethanol water extract of roots inhibit the growth of Mycobacterium tuberculosis. It is given internally in pthisis and scrofulous affections (Srinivasan. et al., 2007). However, the hypoglycaemic and antidiabetic potentials of this shrub have not been scientifically evaluated despite the extensive use of the plant root in the management of diabetes in traditional medicine (Kirtikar and Basu, 1999). The present study was, therefore, designed to evaluate the dose-dependent hypoglycaemic effect of the methanolic root extract in normal, glucose fed hyperglycaemic and streptozotocin- induced diabetic rats.

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Materials and methods

Plant material

The root of *Caesalpinia digyna* Rottler was purchased in June 2007 from abirami botanicals of tuticorin, Tamilnadu, India, and was identified and authenticated by resident botanist, Prof. Dr.S.Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamilnadu, India.

Methanol extract preparation

The root was chopped to small pieces and dried in shade. The dried root was powdered and a weighed quantity of the powder (890 g) was passed through sieve number 20 and subjected to hot solvent extraction in a soxlet apparatus using methonal, at a temperature range of 60-70°C. Before and after every extraction the marc was completely dried and weighed. The extract was concentrated to dryness at 40°C under reduced pressure in a rotary vacuum evaporator. The methanolic root extract of *Caesalpinia digyna* Rottler (MECD) yielded brown semi-solid residue, weighing 7.0g (7.0%) and the extract was preserved in a refrigerator till further usage.

Animals

Inbred adult wistar albino rats (150-280 g) of either sex were obtained from animal house of C.L.Baid Metha College of Pharmacy, Chennai Tamilnadu, India. The animals were maintained in a well-ventilated room with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet fed and tap water was provided *ad libitum* through out experimentation period. Animals were acclimatized to laboratory conditions one week prior to initiation of experiments. Fasting refers to that the animals were deprived of food for 16 hours but were allowed to free access for water.

Determination of blood glucose levels

Blood was collected from tip of the tail vein and fasting blood glucose level (mg/dl) was measured using single touch glucometer (Ascensia ENTRUST, Bayer) based on glucose oxidase method.

Study on normoglycaemic and glucose - hyperglycaemic rats [NG-OGTT]

A combined methodology is preferred for the activity assessment of extract in order to avoid wasting animals; there are some modifications incorporated in the time pattern for blood glucose level determination. After overnight fasting (16 h) the blood glucose level of rats were determined and then were given the test samples and standard. The animals were divided in to four groups of 6 rats in each and treated orally at specific time intervals. Group I - Normoglycaemic control (0.5% CMC 5ml/kg), Group II - Standard control (glibenclamide 0.4mg/kg), Group III and Group IV- Test control (MECD of 250 and 500mg/kg)

Test samples and standard were given immediately after the collection of initial blood samples. The blood glucose levels were determined in the following pattern: 30 and 60 min to access the effect of test samples on normoglycaemic animals. The rats were then loaded orally with 2g/kg glucose and the glucose concentrations were determined at 60, 90 and 210 min after glucose load (Mustafa Aslan et al., 2007). In the present study, glibenclamide (0.4 mg/kg) was used as the standard drug (Rahul Somani et al., 2006).

Study on diabetic rats

Induction of diabetes

Adult inbred wistar albino rats (32 numbers) of either sex were over night fasted and received a freshly prepared solution of streptozotocin (STZ), [Sigma Chemical Co, St Louis, MO, USA], (45 mg/kg) in 0.1 M sodium citrate buffer, PH 4.5, injected intraperitoneally in a volume of 1 ml/kg. After injection the animals had free access to food and water and were given 5% glucose in their drinking water for the first 24 hours to counter any initial hypoglycemia. Normal rats (6 numbers) received 1ml citrate buffer as vehicle. The development of diabetes was confirmed after 48 hours of the streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dl were selected for the experimentation (pari et al., 2004.)

Acute antidiabetic effect of test samples

The animals were divided into 4 groups each constituting 6 STZ induced diabetic rats. Group I – Disease control (0.5% CMC 5ml/kg/p.o), Group II – Standard control (glibenclamide 0.4mg/kg/p.o), Group III and Group IV- Test control (MECD of 250 and 500mg/kg/p.o).

In a single dose treatment study, all surviving diabetic animals were fasted overnight. Blood samples were collected from the fasted animals prior to the treatment with above dosage schedule and after drug administration at 0, 2, 4, and 6 hour time interval to determine the blood glucose level by glucometer. (Rahul Somani et al., 2006).

Statistical analysis

Values are presented as means \pm S.E.M. Statistical difference between the treatments and the controls were tested by one-way analysis of variance (ANOVA) followed by Dunnett's test using 7.5 version of SPSS computer software. The values were considered significant when $P < 0.05$.

Results

Effect of MECD on blood glucose levels in normoglycaemic and glucose induced hyperglycaemic rats. [NG-OGTT]

The effect of MECD on blood glucose levels of normoglycaemic plus glucose-hyperglycaemic (NG-OGTT) is shown in table 1. MECD at both doses did not exhibit significant hypoglycaemic effect in fasted normal rats after 30 minutes of administration. A high dose of 500mg/kg reduced blood glucose level more significantly ($P < 0.01$) compared to the low dose of 200mg/kg ($P < 0.05$) after 60 min of drug administration. In the same rats which are loaded with glucose (2gm/kg) after 60 min of drug administration a low dose of 250mg/kg reduced blood glucose level with less significance ($P < 0.05$) but a high dose of 500mg/kg/b.w reduced blood glucose more significantly ($P < 0.01$). The standard drug glibenclamide (0.4 mg/kg) treatment showed significant ($P < 0.01$) reduction in blood glucose levels in both normal and glucose induced hyperglycaemic rats ($P < 0.01$).

Acute effect of MECD on blood glucose level in STZ induced diabetic rats

The immediate effect of MECD was shown in Table 2. The acute effect of MECD was evaluated by the oral administration of 250mg/kg and 500mg/kg at the 2nd, 4th and 6th hour of acute study. Two concentrations of the extract (250mg and 500mg/kg) did not produce significant reduction in the blood glucose levels in STZ induced diabetic rats. Only at the 6th hour of administration dose level of 500mg/kg and of standard (glibenclamide 0.4mg/kg) showed significant reduction in blood glucose levels of STZ induced diabetic rats ($P < 0.01$).

Discussion

The present paper discussed about the antidiabetic effect of the methanolic root extract of *Caesalpinia digyna* on glucose induced hyperglycaemic and streptozotocin induced diabetic rats. The MECD at doses 250 and 500 mg/kg did not significantly suppress blood glucose levels in overnight fasted normoglycaemic animals but showed significant improvement in glucose tolerance in glucose fed hyperglycaemic normal rats. Such an effect may be accounted for, in part, by a decrease in rate of intestinal glucose absorption, achieved by an extra pancreatic action including stimulation of peripheral glucose utilization or enhancing glycolytic and glycogenic process. (Mukherjee and Soha et al., 1997) In acute study, a higher dose of MECD 500mg/kg showed significant result in the reduction of blood sugar level at 6th hour of administration.

Conclusion

Based on the obtained results and observations, we can infer that the root of the plant under study *Caesalpinia digyna* could be used for the treatment of diabetes mellitus. Longer duration studies of *Caesalpinia digyna* (Rottler) root and its isolated compounds on chronic models are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

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Table 1: Effect of methanolic root extract of *Caesalpinia digyna* Rottler (MECD) on blood glucose in normal and 2g/kg of glucose-loaded hyperglycaemic [NG-OGTT] rats

Groups	Test Sample (mg/kg)	Blood glucose levels (mg/dl)					
		0 min	30 min	60min (glucose load)	120min	150min	270min
I	Control	74.6±2.5	80.8±3.2	76.5±2.4	126.8±4.3	100.6±5.4	78.5±2.5
II	Std-0.4	74.3±2.2	51.6±2.8**	42.0±3.4**	90.1±5.2**	71.0±4.8**	56.0±3.6**
III	MECD-250	66.3±1.4	70.6±4.1 ^{ns}	62.6±4.5*	122.9±3.9 ^{ns}	82.1±3.8*	62.8±5.0*
IV	MECD-500	74.0±2.5	72.4±5.2 ^{ns}	59.2±2.5**	110.0±4.2*	75.2±5.1**	60.3±4.7**

The blood glucose values of group II, III and IV are compared with control animal values. * $P < 0.05$, ** $P < 0.01$, ns-non significant

Table 2: Effect of acute treatment of methanolic root extract of *Caesalpinia digyna* Rottler (MECD) on blood glucose in STZ induced diabetic rats.

Groups	Blood glucose levels (mg/dl)			
	0 hour	2 nd hour	4 th hour	6 th hour
Group I	245.83±5.0	249.72±3.8 ^{ns}	248.00±5.3 ^{ns}	250.16±3.8 ^{ns}
Group II	230.20±5.2	224.00±4.6 ^{ns}	219.00±3.7 ^{ns}	204.12±2.9**
Group III	231.43±4.3	230.32±5.1 ^{ns}	223.00±4.1 ^{ns}	210.00±3.9**
Group IV	229.36±3.1	222.70±3.5 ^{ns}	220.00±2.7 ^{ns}	206.40±5.3**

The blood glucose values of all groups at 2nd, 4th and 6th hour are compared with their zero hour values. ** $P < 0.01$, ns-non significant.

References

- [1] Anon., The wealth of India, Vol.3. CSIR, New Delhi, India, 1992, 12-13
- [2] Kritikar KR, Basu BD, Indian medicinal Plants, Vol.2. International Book Sellers and Publishers, Dehradun, India, 1999, 851.
- [3] Mukherjee PK, Saha K, Pal M, Saha BP, Effect of *Nelumbo nucifera* rhizome extract on blood sugar level in rats. Journal of Ethnopharmacology, 58, 1997, 207-213.
- [4] Mustafa A, Didem D, Orhan N, *In vivo* antidiabetic and antioxidant potential of *Helichrysum plicatum ssp. plicatum capitulum* in streptozotocin-induced-diabetic rats. Journal of Ethnopharmacology, 109, 2007, 54-59.
- [5] Pari L, Latha M, Sandhya Sitasawad, Ramesh Bhonde, Insulin –secretagogue activity and cytoprotective role of the traditional Antidiabetic plant *Scoparia dulcis* (Sweet Broomweed). Life sciences, 75, 2004, 2003-2014.
- [6] Rahul Somani, Sanjay Kasture, Abhay Kumar Singhai, Antidiabetic potential of *butea monosperma* in rats. Fitoterapia, 77, 2006, 80-90.
- [7] Ronald Khan, George K, Allanc Moses, JOSLIN'S DIABETES MELLITUS. Elsevier Publication, 14th Edition, 1995, 12.
- [8] Srinivasan R, Chandrasekar MJN, Nanjan MJ, Suresh B, Antioxidant activity of *Caesalpinia digyna* root. Journal of Ethnopharmacology, 113(2), 2007, 284-291
