Hypoglycaemic and Hypolipidemic Activity of Methanolic Root Extract of *Caesalpinia Digyna* (Rottler) in Streptozotocin-Induced Diabetic Rats

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Summary

The methanolic root extract of Caesalpinia digyna Rottler (MECD) was tested for its hypoglycaemic and hypolipidemic activity by sub 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 0^{th},10^{th} and 15^{th} day and biochemical parameters like glycosylated haemoglobin, serum protein, serum creatinine and lipid profile were estimated after 14 days of sub acute study. The results revealed that in sub acute treated groups administration of a low dose of 250mg/kg reduced blood glucose level with less significance ($p<0.05$) but a high dose of 500mg/kg reduced blood glucose significantly ($p<0.01$).In addition, changes in body weight, glycosylated haemoglobin, serum protein, serum creatinine and serum lipid profile levels assessed in the extract treated diabetic rats were compared with standard control, diabetic control and normal animals in sub acute treated groups. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the indigenous system of medicine.

***Key words*:** *Caesalpinia digyna* root; Hypoglycaemic activity; Hypolipidemic activity; Streptozotocin; Lipid profile.

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Introduction

Caesalpinia digyna Rottler (Family:Leguminosae) is a large, scadent, 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 (1) Chemical investigations of the plant have shown the presence of caesalpinine A, cellallocinnine, 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 (2). 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 (3). The present study was, therefore, designed to evaluate the dose-dependent hypoglycaemic and antidiabetic effects of the methanolic root extract in normal, glucose fed hyperglycaemic and streptozotocin- induced diabetic rats.

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 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 intraperitonially 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.) In the present study, glibenclamide (0.4 mg/kg) was used as the standard drug (4).

Subacute antidiabetic effect of test samples

The animals were dived in to 5 groups .Group I consists of normoglycaemic rats and the remaining 4 groups consisted of 6 STZ induced diabetic rats. The mentioned groups were treated orally as follows: Group I -Normal control (0.5% CMC 5ml/kg), Group II-Disease control (0.5% CMC 5ml/kg), Group III-Standard control (glibenclamide 0.4mg/kg) Group IV and Group V-Test control (MECD of 250 and 500 mg/kg).

The above mentioned treatment schedule was followed for the respective group of animals for 14 days. Body weight changes were measured for overnight fasted animals on 0^{th}, 10^{th} and 15^{th} day of study and the blood samples were collected at the same days to estimate blood glucose levels using glucometer.(5)

At the end of the study, all the animals were sacrificed under light ether anaesthesia.The rats were sacrificed by decapitation and blood was collected by bleeding of carotid artery and serum was separated to study various biochemical parameters like glycosylated haemoglobin(Excel diagnostics pvt Ltd),serum protein(Beacon diagnostics Ltd), total cholesterol(Span diagnostics Ltd),HDL-cholesterol(Span diagnostics Ltd),triglycerides(Span diagnostics Ltd),LDL-cholesterol (6) ,VLDL-cholesterol (7). The relevant organs like liver and pancreas were removed dissected out and washed with ice-cold saline. The pancreatic tissues were preserved in bouin's fluid for histopathological studies.

Statistical analysis

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

Results

Sub-acute effect of MECD on body weight and blood glucose level in STZ induced diabetic rats

The MECD at oral dose level of 250mg/kg do not show significant improvement in the body weight of STZ induced diabetic rats up to the 10^{th} day of treatment and showed a slight significance in the body weight improvement on 15^{th} day ($P<0.05$). An oral dose of 500mg/kg and standard drug glibenclamide (0.4mg/kg) shows significant ($P<0.01$) improvement in the body weight of STZ induced diabetic rats on 10^{th} day and 15^{th} day of treatment. Treatment with MECD 250mg/kg showed less significant result ($P<0.05$) in the reduction of the blood glucose level on 10^{th} day compared to a high dose of 500mg/kg and standard ($P<0.01$). Treatment with MECD of both doses and standard produced a significant ($P<0.01$) drop in blood glucose level on 15^{th} day of Subacute study. Results are shown in Table 1.

Sub-acute effect of MECD on biochemical parameters in STZ induced diabetic rats

The diabetic control rats showed significant increase in the glycosylated haemoglobin (GHb%), total cholesterol(TC),triglycerides(TG),low density lipoproteins(LDL),very low density lipoproteins(VLDL),serum creatinine and a significant decrease in serum total protein and high density lipoprotein (HDL) levels when compared to normal control rats .GHb % level in MECD (250mg and 500mg/kg) treated diabetic rats decreased less significantly ($P<0.05$) compared to that in rats treated with standard ($P<0.01$). The serum total protein levels in MECD treated diabetic rats with doses of 250mg and 500mg/kg showed significant [($P<0.05$) & ($P<0.01$)] increase respectively.Glibenclamide (0.4mg/kg) also showed significant ($P<0.01$) results.

Serum total cholesterol levels of diabetic animals treated with both the doses of MECD (250 and 500mg/kg) and standard showed significant ($p<0.01$) decrease in cholesterol level when compared to disease control group. Diabetic rats treated with standard and MECD of dose 250mg/kg showed less significance ($P<0.05$) in the reduction of serum HDL levels compared to that of those treated with MECD of dose 500 mg/kg ($P<0.01$).Triglycerides and LDL levels of diabetic rats treated with MECD of 250mg/kg were reduced less significantly ($P<0.05$) compared to that of rats treated with MECD of 500mg/kg and standard ($P<0.01$). In diabetic rats treated with MECD (250 and 500mg/kg) and standard, VLDL and serum creatinine levels were decreased significantly ($P<0.05$) compared to that of disease control group. Results are shown in Table 2.

Table 1: Effect of sub acute treatment of methanolic root extract of *Caesalpinia digyna* Rottler (MECD) on body weight changes and blood glucose level in STZ induced diabetic rats

Group	Treatment	Dose (Kg^{-1} Body Weight)	Parameter Gm &mg/dl	Day of measurement		
				O Day	10^{th} Day	15^{th} Day
I	Control(0.5% SCMC)	5 ml	Body weight Blood glucose	192.45 ± 1.24 77.37 ± 1.6	196.92 ± 1.2 79.43 ± 2.5	201.5 ±1.5 82.5 ± 11.5
II	Disease control (STZ)	45mg	Body weight Blood glucose	210.24 ±0.99 245.83 ± 5.1	167.89 ±1.4** 267.0 ± 6.9**	153.5 ±0.7** 288.3 ± 5.3**
III	Standard (Glibenclamide+STZ)	0.4mg	Body weight Blood glucose	183.13 ± 2.64 230.0 ± 5.2	185.47±3.2* 167.30 ± 4.5**	187.9 ±1.5** 107.60 ± 3.2**
IV	Test I (MECD+STZ)	250mg	Body weight Blood glucose	197.62 ± 4.3 231.43 ± 4.3	166.6±5.4^{ns} 180.16 ± 3.78*	173.20± 4.2* 133.16 ± 4.7**
V	Test II (MECD+STZ)	500mg	Body weight Blood glucose	189.92 ± 1.7 229.36 ± 3.1	175.60 ±1.3* 170.33 ±4.6**	187.60± 4.1** 117.17 ± 5.3**

Group II is compared with Group I .Groups III, IV, V are compared with groupII.**$P<0.01$,*$P<0.05$,ns- non significant

Table 2: Effect of sub acute treatment of methanolic root extract of *Caesalpinia digyna* Rottler (MECD) on various biochemical parameters in STZ induced diabetic rats

Group	Treatment	Dose (kg^{-1} body weight)	Glycosy-lated haemoglobin (GHb%)	Serum total protein (mg/dl)	Total Cholesterol (mg/dl)	HDL mg/dl)	Trigly cerides (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Creatinine (mg/dl)
I	Control(0.5 % SCMC)	5 ml	7.217 ± 0.7575	6.505± 0.1469	118.9 ± 5.4	41.99 ± 2.6	142.0 ± 3.5	48.52 ± 5.0	28.40 ± 0.69	0.7317 ± 0.046
II	Diabetic control(STZ)	45mg	12.950 ± 0.393**	3.513±0.1 304**	167.4 ± 3.61**	27.37 ± 1.9**	188.0 ± 3.9**	102.50 ± 5.3**	37.61± 0.77**	1.732 ± 0.053**
III	Standard (Glibencla mide + STZ)	0.4mg	7.980 ± 0.6566**	5.920±0.2 503**	120.7 ± 3.80**	36.75 ± 3.0*	152.0 ± 2.9**	55.54 ± 5.2**	32.41 ± 0.60*	1.242 ± 0.038*
IV	Test I (MECD + STZ)	250mg	8.645 ± 0.5022*	5.118± 0.5430*	126.3 ± 3.46**	33.74 ± 3.3*	168.6 ± 3.2*	83.36 ± 3.5*	33.72 ± 0.64*	1.367 ± 0.035*
V	Test II (MECD + STZ)	500mg	8.222 ± 0.5910*	5.428±0.5 525**	140.8 ± 4.167**	38.03 ± 2.4**	150.6 ± 3.0**	55.87 ± 3.0**	32.44 ± 0.60*	1.273 ± 0.045*

Group II is compared with group I and Group III, IV and V are compared with Group II. $^{**}P<0.01$ $^{*}P<0.05$

Discussion

The present paper discussed about the antidiabetic effect of the methanolic root extract of *Caesalpinia digyna* on streptozotocin induced diabetic rats In the sub-acute study, induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting (8) and due to loss of proteins (9).Diabetic rats treated with the MECD showed an increase in body weight as compared to the diabetic control, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis. Glibenclamide treatment brought down the sugar levels from the first day of the treatment. MECD (250 and 500mg/kg) treatment produces significant reduction in blood glucose levels from 10^{th} day of treatment and a steady decrease was observed there after.

Increased non-enzymatic and autooxidative glycosylation is one of the possible mechanisms linking hyperglycaemia and vascular complications of diabetes. In the present study diabetic rats had shown higher levels of HbA_1 compared to those in normal rats indicating their poor glycaemic control (10). Treatment with MECD, showed a significant decrease in HbA_1 levels in diabetic rats. This property provides a practical and

objective means of assessing average blood glucose levels over a time frame of about 2 months and has proven to be a very useful adjunct to self monitoring of blood glucose (SMBG) (11).Excessive break down of body protein in conjunction with either inadequate supply or defective utilization observed in uncontrolled diabetes may be accompanied by hypoalbumiemia (12). MECD seems to resort this effect due to the hypoglycaemic status.

Myocardial infarction, caused by atherosclerosis of the coronary arteries, is the most common caused of death in diabetics (13). Hypercholesterolemia and hypertriglyceridemia have been reported to occur in diabetic rats (14-16). It is well known that the level of glycaemic control is the major determinant of serum level of very low density lipoprotein (VLDL).Several investigations demonstrated that near normalization of the blood glucose level resulted in significant reductions in levels of plasma cholesterol, trigltcerides, free fatty acids and plasma protein (10). In the present study elevated serum total cholesterol, triglycerides, LDL-levels, VLDL-levels and reduced HDL levels were observed in STZ-induced diabetic rats. MECD (250mg/kg and 500 mg/kg) treatment in diabetic animals produced beneficial improvement in the lipid profile which showed a hypolipidemic effect in diabetic rats. Increased serum creatinine in diabetic rats indicates cardiac muscular damage (17). Elevated concentrations of serum creatinine were recovered by the treatment with MECD of both concentrations and standard glibenclamide suggesting their cardio protective effect.

Histopathological studies that showed prominent islets cell hyperplasia and regeneration of islet cell show a proof for the possible antidiabetic property of the root extract of *Caesalpinia digyna.* (Fig. 1-5)

Conclusion

From this we can state that methanolic fraction of Rottler root has beneficial effects on blood glucose levels as well as improving hyperlipidaemia due to diabetes through its hypolipidemic action. Further studies are required to establish the hypoglycaemic activity of *Caesalpinia digyna* in terms of molecular mechanism(s) involved in the activity.

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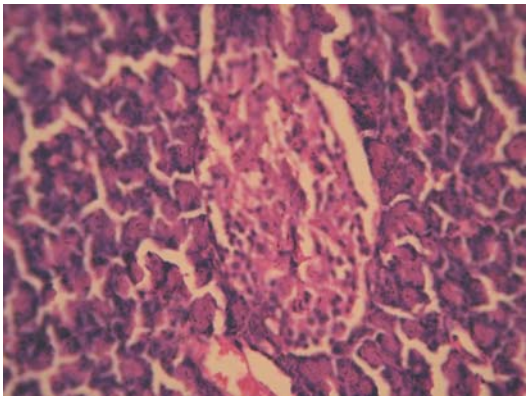
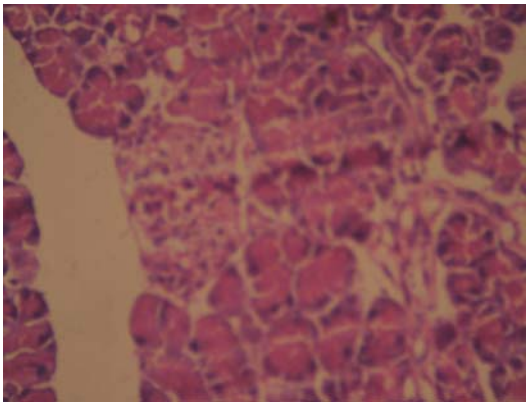
Fig. 1



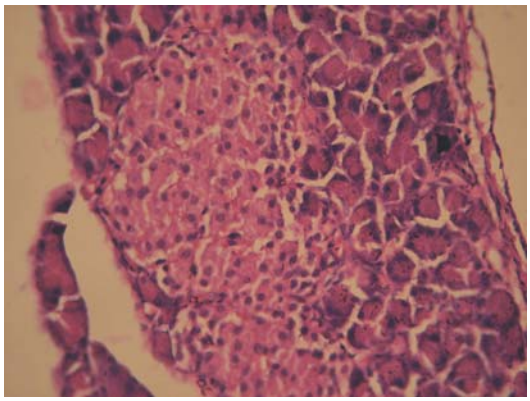
Pancreas of normal control showing normal appearance of islets cells.

Fig. 2



Pancreas of diabetic control shows damaged and atrophic islets with acini

Fig. 3



Pancreas of standard group

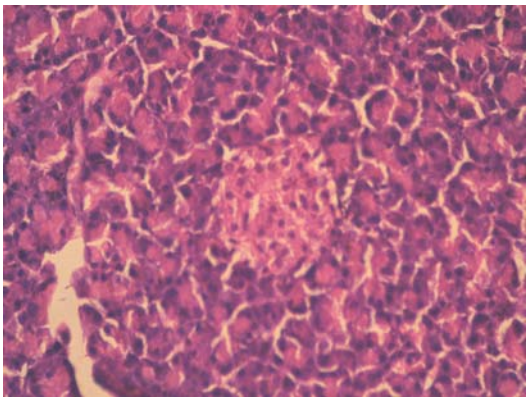


Fig.4

Pancreas of treated group(MECD 250mg/kg) showing small islet cells.

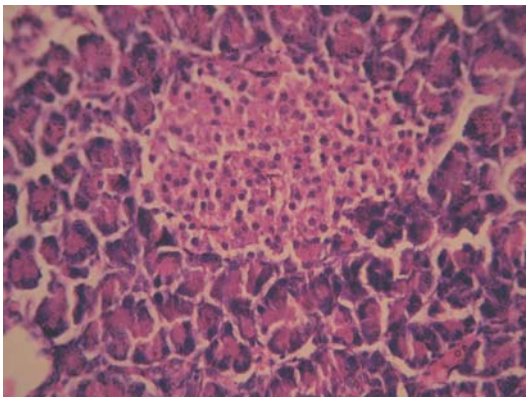


Fig. 5

Pancreas of treated group(MECD 500mg/kg) showing hyperplastic islets with acini

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