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Evaluation of anti-diabetic activity on ethanolic extract of *Delonix regia* seeds

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Abstract
The present study was performed to investigate the local ethno medical claim scientifically by screening the acute hypoglycaemic effect of *Delonix regia* seeds in normal and streptozotocin induced diabetic mice. It has also significantly improved the glucose tolerance in normal mice. The study comprises the evaluation of antidiabetic activities of *Delonix regia* belonging to Leguminosae family. Initially, *In vitro* α-amylase inhibitor activity was performed as a preliminary screening for petroleum ether, ethyl acetate, ethanol and aqueous extracts of the plant. In comparison with all the extracts, ethanol extracts shown promising activity with IC_{50} values of 550.29µg/ml for *D. regia* respectively. Hence EEDR were further selected for DPPH radical scavenging activity. *Delonix regia* resulted in 455µg/ml in DPPH radical scavenging activity. Hence, EEDR was selected to evaluate *In vivo* Antidiabetic activity.

Keywords: Delonix regia, streptozotocin, oral glucose tolerance, glucometer, ethanolic extract

Introduction
The incidence of diabetic is growing rapidly in United States and worldwide. Globally as of 2010, an estimated 285 million people had diabetes [1]. Type 1 diabetes is due to the autoimmune destruction of beta cells of pancreas and type 2 diabetes is because of the pancreas produces insufficient amount of insulin or body cells becomes resistant towards insulin. Type 1 diabetes accounts for 5-10% whereas type 2 diabetes is much more common and accounts for 90-95% cases [2]. Diabetes mellitus (DM) is a major public health problem in the developed as well as developing countries. India has been projected by WHO AS the country with fastest growing population of DM [3].

The presence of variety of effective synthetic drugs, use of medicinal plants for maintaining human health has acquired a lot of importance in the present era [4]. Herbal medicines are particularly used by the traditional practitioners since the ancient time but they do not have scientific data. The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare. Nowadays person prefers plant based medicines over synthetic medication for the treatment of different disease because of their safety as well as economy [5]. *Delonix regia* is a plant from the family Leguminosae, is extensively cultivated in most regions of the world. Sometime known as royal Poinciana may flower plant or flamboyant, many branched, broad, spreading, flat crowned deciduous tree and well known for its brilliant display of red-orange bloom, literally covering the tree from May to June [6]. The Delonix regia will provide fullest flowering and best growth when planted in full sun location [7].

Objective
To investigate the folk claim use in hypoglycaemic effect of *Delonix regia* seeds in streptozotocin induced diabetic mice by oral glucose tolerance test by glucometer and hypolipidemic activity.

Materials and methods
Plant material
Seeds of *Delonix regia* were collected from Nungambakkam, Chennai and the plant was authenticated by Dr. M. Palanisamy, Scientist 'D'- In – Charge, Botanical Survey of India, Southern Regional Centre, T. N. A. U Campus, Lawley Road, Coimbatore-641003. The specimen copy of this plant is preserved in the Department of Pharmacognosy, Madras Medical College, Chennai-600003. Herbarium to future reference. The voucher number is 14/PCOG/2015.

Preparation of EEDR extract
The seeds were collected from seed pods by applying mechanical strength and spreader on a neat sheet for shade drying and powdered using mechanical grinder. The powdered seeds of *D. regia* were stored in an air tight container. The coarsely powdered seeds were subjected to successive solvent extraction using ethanol (90%). The extract were concentrated under reduced pressure and stored in desiccators until further use and the percentage e yield of corresponding extracts were calculated.

Pharmacological study
Acute toxicity study
During preliminary toxicity study, no adverse effect or mortality was observed in albino mice with oral administration by fixed dose method of OECD guideline 425. The EEDR were administered to groups of 6 mice by oral route up to high dose of 2000mg/kg and mortality was observed after 24hours. No adverse effects on the health, growth, and organ weight ratio, hematological and clinical chemistry parameters. It may be concluded that the LD_{50} of the seeds is greater than 2000 mg/kg. Mortality, clinical observation, body weight & gross necropsy findings were evaluated [8].

Animals
Healthy Wistar albino male rats (weighing 170-230mg) were used. The animals were kept clean and dry polycarbonate cages and maintained in a well-ventilated animal house with 12 hour light / 12 hour dark cycle. Animals were fasted overnight before commencing the experiment, but had free access to water [9].

Chemicals
Streptozotocin was obtained from Lab chemicals, India, Glibenclamide was obtained from Aventis pharmaceuticals, Goa.

Standard drug
Glibenclamide drug was used as a standard drug. Streptozotocin monohydrate 60mg/kg body weight was dissolved in 0.9% v/v Cold normal saline and injected intraperitoneally to 18 hours fasted rats (24 no's,).

Determination of blood glucose level
Blood glucose level was monitored by using Glucometer.

Oral glucose tolerance test
Glucose 2g/kg fed 30 minutes prior to the administration of extracts. Blood withdrawn from the retro orbital sinus at 30, 60 and 120minutes of extract administration and the plasma obtained after centrifugation at 3000rpm was estimated for fasting plasma glucose level using a glucose oxidase-peroxidase glucose estimation kit [10].

Induction of non-insulin dependent diabetes mellitus (NIDDM)
Streptozotocin induced diabetes
The rats were injected intraperitoneally with Streptozotocin dissolved in citrate buffer (P^H 4.5) at a dose of 45mg/kg body weight. Hyperglycaemia was confirmed by the elevated blood glucose level in plasma, determine at the end of 0 hr, 1hr, 3hr, 5hr, 7hr, 24hr Acute study and then 7 day after injection. Rats with blood glucose level above 126mg/dl were selected for the study. Samples are analysed for blood glucose content by Glucometer. Blood samples were collected by tail clipping method. Rats with blood glucose level of greater than 250mg/dl were considered diabetic and were selected for study (WHO, 1985) Rats were divided randomly into 5 Groups of 6 rats per group for screening. Group II-IV in order to induce hyperglycaemia in experimental Wistar rats (170–230 g body Weight (b/w) and the six control rats (group-I) received equal volume of 0.9% v/v Cold normal saline injected intraperitoneally [9, 10].

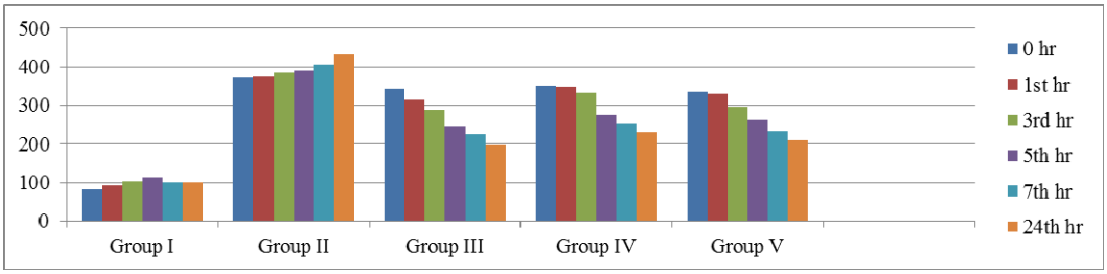
Results

Table 1: The Animals received treatments

S. No	Group	Name of the drug	Dose	No of animals	Duration of dosage (days)
1	Group – 1	Normal control	Saline	6	28
2	Group – 2	Diabetic control (0.9% v/v saline)	2ml p.o	6	28
3	Group – 3	Standard (glibenclamide)	3mg/kg p.o	6	28
4	Group – 4	Test drug – I	200mg/kg p.o	6	28
5	Group – 5	Test drug – II	400mg/kg p.o	6	28

Table 2: Effects of EEDR On blood glucose level in streptozotocin induced diabetic rats (mg/dl)-acute study

Treatment	0 hr	1st hr	3rd hr	5th hr	7th hr	24th hr
Group I	83±1.3	93±1.6	102±1.2	112±1.8	99±1.1	101±1.9
Group II	372±0.4	374±1.1	385±0.2	390±1.5	407±1.8	431±2.1
Group III	342±1.2	315±1.4	288±1.7	246±2.3*	225±1.2*	198±1.8*
Group IV	352±2.1	348±2.2	332±2.1	276±1.3*	252±1.6*	232±1.6*
Group V	334±1.4	329±0.8	297±1.3	262±2.3*	233±2.2*	210±2.3*



Group I compared with Groups II, III, IV, V
Group II compared with Groups III, IV, V

Fig 1: Graphical representation of blood glucose level in study groups-acute study

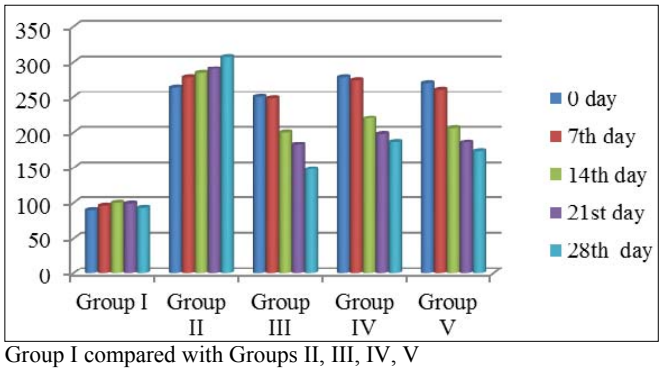
Table 3: Effects of EEDR On blood glucose level in streptozotocin induced diabetic rats (mg/dl)-chronic study

Treatment	0 day	7th day	14th day	21st day	28th day
Group I	90±1.4	96±1.3	100±1.2	99±1.2	93±1.7
Group II	264±1.4	278±1.6	284±0.8	290±1.1	307±1.9
Group III	250±0.9	248±0.5	200±1.3*	182±1.3*	148±1.2*
Group IV	278±1.3	274±2.7	220±1.9*	198±1.3*	187±1.5*
Group V	270±1.2	261±2.3	206±1.5*	186±2.5*	173±1.6*

One way ANOVA values are expressed as mean ±SD n = 6
*P < 0.05 compared to diabetic control

Note:

Group I	Normal control
Group II	Diabetic control
Group III	Standard drug (Glibenclamide 3mg/kg)
Group IV	Ethanolic extract of *Delonix regia* 200mg/kg
Group V	Ethanolic extract of *Delonix regia* 400mg/kg



Group I compared with Groups II, III, IV, V
Group II compared with Groups III, IV, V

Fig 2: Graphical representation of blood glucose level in study groups-chronic study

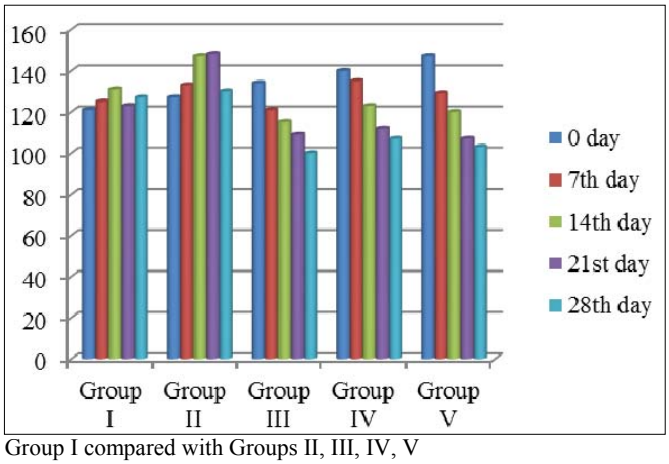
Table 4: Changes in Body Weight (grams)

Treatment	0 day	7th day	14th day	21st day	28th day
Group I	121±6.68	125.67±6.68	131.67±6.68	123.67±6.68	127.67±6.68
Group II	127.12±5.51	133±6.33	147.38±3.25	148.26±3.25	130.57±4.15
Group III	134.28±4.11	121.01±3.18	115.47±1.71*	109.19±6.15*	100.87±7.54*
Group IV	140.51±3.64	135.47±5.48	123.24±2.31*	112.35±8.48*	107.95±3.18*
Group V	147.17±9.47	129.17±3.49	120.54±5.47*	107.68±4.67*	103.91±1.26*

One way ANOVA values are expressed as mean ±SD (n = 6)
*P < 0.05 compared to diabetic control

Note:

Group I	Normal control
Group II	Diabetic control
Group III	Standard drug (Glibenclamide 3mg/kg)
Group IV	Ethanolic extract of *Delonix regia* 200mg/kg
Group V	Ethanolic extract of *Delonix regia* 400mg/kg



Group I compared with Groups II, III, IV, V
Group II compared with Groups III, IV, V

Fig 2: Graphical representation of changes in body weight (grams)

Effect of ethanolic extract of *Delonix regia* (Boojer. Hook.). Raf. seeds on plasma lipid profile

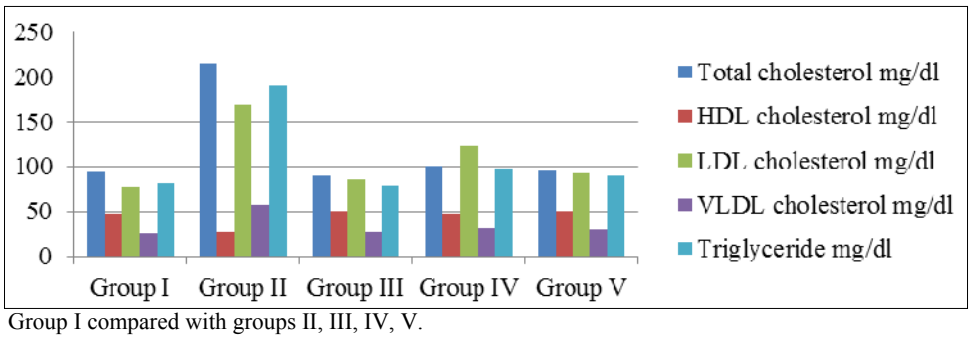
Table 5: Plasma lipid profile

Treatment	Total cholesterol (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	Triglyceride (mg/dl)
Group I	94.66±5.8	48.00±4.17	77.40±4.46	26.50±2.58	81.16±6.24
Group II	215±4.84	27.50±3.30	169.00±5.51	58.83±4.57	190.50±9.62
Group III	90.50±12.78	51.83±3.97	85.83±4.44*	27.66±4.45*	79.17±4.44*
Group IV	101.83±4.62	48.00±4.51	123.83±5.87*	31.66±4.45*	96.16±5.45*
Group V	95.33±5.27	50.16±4.79	92.16±6.01*	29.66±4.45*	90.17±4.91*

One way ANOVA values are expressed as mean ±SD n = 6
*P < 0.05 compared to diabetic control

Note:

Group I	Normal control
Group II	Diabetic control
Group III	Standard drug (Glibenclamide 3mg/kg)
Group IV	Ethanolic extract of *Delonix regia* 200mg/kg
Group V	Ethanolic extract of *Delonix regia* 400mg/kg



Group I compared with groups II, III, IV, V.
Group II compared with groups III, IV, V.

Fig 2: Graphical representation of lipid profile in study groups

Conclusion

The present study concludes that EEDR seeds exhibited the antidiabetic effect of ethanolic extract at the dose of 200mg/kg and 400mg/kg produced a dose dependent hypoglycaemia on streptozotocin induced diabetic rats. It is clear from the data that the blood glucose levels of control diabetic animals continued to increase whereas extract treated diabetic rats showed significantly reduced levels. Maximum reduction of blood glucose level was achieved by the ethanolic extract, 400mg/kg from 0 hours to 24 hours respectively, whereas ethanol extract at the dose of 200mg/kg showed a slightly lower effect. Significant level of $p<0.01$. Glibenclamide showed maximum reduction after 24 hours with a significant level of $p<0.0$. At the end of 28th day, ethanolic extracts produced a significant reduction of body weight of 67% at 200mg/kg and 76% at 400mg/kg compared to diabetic control.

On the other hand, Glibenclamide produced a significant blood glucose reduction of 65% and the ethanol extracts brought about 54% at 200mg/kg and 57% at 400mg/kg. EEDR of *Delonix regia* seeds and glibenclamide significantly depressed the peak of blood glucose level at 2 hours. After glucose loading with a significant level of $p<0.01$.

Statistical analysis

Results are expressed as Mean±SD and analysed statistically by one way ANOVA followed by Dunnett's multiple comparison testing. All the experimental results were compared with control group. $**p<0.01$, very significant; $*p<0.05$, significant.

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