

Hypoglycemic Effect of *Calotropis gigantea* Linn. Leaves and Flowers in Streptozotocin-Induced Diabetic Rats

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Received: 03 Dec 2010 / Accepted: 11 Feb 2011
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Abstract

Objectives: To evaluate the hypoglycemic and anti-diabetic activity of chloroform extract of *Calotropis gigantea* leaves and flowers in normal rats and streptozotocin induced diabetes.

Methods: The hypoglycemic activity in normal rats was carried out by treatment using chloroform extract of *Calotropis gigantea* leaf and flower 10, 20 and 50 mg/kg, orally. The oral glucose tolerance test was carried out by administering glucose (2 g/kg, p.o), to non-diabetic rats treated with leaf and flowers extracts at oral doses 10, 20 and 50 mg/kg, p.o and glibenclamide 10 mg/kg. The serum glucose was then measured at 0, 1.5, 3 and 5 hr after administration of extracts/drug. Streptozotocin-induced diabetic rats were administered the same doses of leaf and flower extracts, and standard drugs glibenclamide was given to the normal rats or 0.5 ml of 5% Tween-80, for 27 days. The blood sample from all groups collected by retro-orbital puncture on 7, 14, 21 and 27th days after administration of the extracts/drug and used for the estimation of serum glucose levels using the glucose kit.

Results: The *Calotropis gigantea* leaves and flowers extracts were effective in lowering serum glucose levels in normal rats. Improvement in oral glucose tolerance was also registered by treatment with *Calotropis gigantea*. The administration of leaf and flower extracts to streptozotocin-induced diabetic rats showed a significant reduction in serum glucose levels.

Conclusion: It is concluded that chloroform extracts of *Calotropis gigantea* leaves and flowers have significant anti-diabetic activity.

Keywords: Diabetes; Antidiabetic; *Calotropis gigantea*; Oral glucose tolerance test; OGTT; Hypoglycemic.

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Introduction

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fats and protein metabolism. As part of the pathogenesis of type 2 diabetes; skeletal muscle, liver, glucose and adipose tissue become resistant to the hormonal effect of insulin, which in turn lead to decreased insulin-mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis.¹ There are an estimated 143 million people in the world with diabetes mellitus and this number will probably double by the year 2030.² Due to the enormous costs of modern treatment for diabetes in developing countries, the use of medicinal plants and their preparation has flourished as an alternative for the control and prevention of the disease.³

Currently, there are several drugs available for treatment of diabetes mellitus but most of them are synthetic drugs and this makes the anti-diabetic treatment costly. In view of reducing the cost of treatment for diabetes mellitus, there is considerable research for herbal production as anti-diabetics and around 12000 plants have been reported to possess anti-diabetic property.⁴ *Swarnabhasma*; an Ayurvedic preparation containing *Calotropis gigantea* R. Br (*C. gigantea*) and flowers were extensively used by Ayurvedic physicians for treatment of disorders such as diabetes mellitus, bronchial asthma, rheumatoid arthritis, and nervous disorders.^{5,6} Chloroform extract of *C. gigantea* leaves and flowers prevent insulin induced resistance in high fructose diet.⁷ Another member of this genus, *Calotropis procera* has also been reported to possess anti-diabetic activity.⁸ *C. gigantea* has been reported to contain proteases, 3'-methylbutanoates of amyryl,⁹ flavonol glycosides,¹⁰ calotropins,¹¹ Stigmasterol and sitosterols,¹² cardenolides,¹³ and pregnanone.¹⁴

Its roots have been used in leprosy, eczema, syphilis, elephantiasis, ulceration, anti-diarrhoeal, and cough in the Indian system of traditional medicine.¹⁵ Antipyretic and analgesic activities,^{11,16} of the alcohol extract of peeled roots of *C. gigantea* were also recently reported in mice models.^{17,18} The milky juice of *C. gigantea* has been reported to be a violent purgative and gastrointestinal irritant and as being used for inducing abortion.¹⁹ The crude latex extract is reported to exhibit strong proteolytic activity, being able to hydrolyse casein, human fibrinogen and crude fibrin clot in a dose dependent manner.²⁰ It has also been reported for Antiamoebic,²¹ wound healing,²² hepatoprotective,²³ and antioxidant,²⁴ properties.

Recently, many natural products containing glycosides, flavonoids and steroids are being reported to possess anti-diabetic and hypoglycaemic effects due to their anti-inflammatory and antioxidant properties.²⁵⁻²⁸ Previous studies carried out on *C. gigantea* have indicated the presence of proteases, glycosides, flavonoids and phenolic compounds and were proven to be responsible for antioxidant and anti-inflammatory properties.^{9-14,17}

Based on these findings, we hypothesized that this plant preparation may also exhibit hypoglycemic and anti-diabetic properties. Therefore, the present study was designed with an objective to evaluate possible anti-diabetic activity of *C. gigantea* using chloroform extract of leaves and flowers in diabetic rats and also to determine improvement in glucose tolerance by using oral glucose tolerance test (OGTT).

Methods

C. gigantea leaves and flowers were collected in the region of Bagalkot-Karnataka India, during September - October 2005. The plant was authenticated at the Dept. of Botany, Basaveswara Science College, Bagalkot-Karnataka India. A voucher specimen (BSC/BOT/04/09) was deposited in the same Institute.

The plant material was shade-dried and uniformly powdered by passing through the sieve #44 and then subjected to hot continuous extraction with petroleum ether (40-60°C) to defat the preparation, followed by chloroform, ethyl acetate and methanol extraction for a 24 hr cycle. The crude extract solution was concentrated by using rotary flash evaporator to produce a semisolid mass, and dried in lyophilizer (Mini Lyotrap, Serial No. J8199/5, LET Scientific LTD, UK).

The Streptozotocin was purchased from Sigma Chemical Co St Louis, USA. Petroleum ether (40-60°C) chloroform, ethyl acetate, and methanol AR grade were purchased from Nice Pvt Ltd, India.

The extracts were then formulated as suspension in distilled water using 5% Tween-80 as suspending agent.²⁹ When different extracts were tested for hypoglycemic activity on normal rats, the chloroform extract was the only effective one and was the one selected for further treatment. The dose ranges were selected based on previous studies.¹⁷ Henceforth, CECGL and CECGF refers to chloroform extracts of *C. gigantea* leaves and flowers respectively.

Wistar albino rats (150-200 g) of either sex were used in this study, after one week period of acclimatization to laboratory conditions. The animals were housed under standard environmental condition of temperature (21 ± 2°C), humidity (51 ± 10%) and 12 hr light-dark cycles with standard pellet diet (Amrut Lab) and water ad libitum. Animals were divided into various groups, each group consisted of three male and three female rats. All the experiments were carried out as per the guidelines of Institutional Animals Ethics Committee (RGE No.821/A/CPCSEA) of College after approval (HSK/IAEC.Clear/2004-2005) dated 27/12/04.

Anti-diabetic activity of chloroform extract of leaves and flower was evaluated using STZ- induced diabetic rats. The STZ was dissolved in citrate buffer (pH 4.5) and was administered by

intraperitoneal route at 55 mg/kg for each rat and their serum glucose levels were estimated after 72 hr. The rats' serum glucose levels which were over and above 300 mg/dl were considered to be diabetic resembling chronic diabetic condition and hence they were selected and divided into eight groups of six rats each.¹

To determine the hypoglycemic activity in normal rats, the rats were kept on fasting for 18 hrs and were divided into seven groups of six rats each.

Group 1: served as the controls, 0.5 ml of 5% Tween-80 in distilled water orally. Tween-80 was used as a suspending agent to suspend the extract for oral administration.

Group 2: CECGL extract 10 mg/kg, p.o

Group 3: CECGL extract 20 mg/kg, p.o

Group 4: CECGL extract 50 mg/kg, p.o

Group 5: CECGF extract 10 mg/kg, p.o

Group 6: CECGF extract 20 mg/kg, p.o

Group 7: CECGF extract 50 mg/kg, p.o

The blood samples from all groups were drawn by retro-orbital plexus from each rat, under anesthesia at 0, 1.5, 3 and 5 hrs after CECGL and CECGF extract administration. The serum glucose levels were estimated from all blood samples using a glucose kit.¹

For the oral glucose tolerance test, the rats were kept on fasting for 18 hrs and then divided into eight groups of six rats each:

Group 1: served as the control, 0.5 ml of 5% Tween-80 in distilled water, p.o

Group 2: glibenclamide 10 mg/kg, p.o

Group 3: CECGL extract 10 mg/kg, p.o

Group 4: CECGL extract 20 mg/kg, p.o

Group 5: CECGL extract 50 mg/kg, p.o

Group 6: CECGF extract 10 mg/kg, p.o

Group 7: CECGF extract 20 mg/kg, p.o

Group 8: CECGF extract 50 mg/kg, p.o

The rats of all groups were loaded with glucose (2 g/kg) 30 minutes after administration of extracts/ drugs. Blood samples were withdrawn from each rat at 0, 0.5, 1.5, and 3 hrs after CECGL and CECGF extract administration and the serum glucose levels were then estimated from all blood samples using a glucose kit^[30, 31]

To determine the anti-diabetic activity, the rats were grouped as follows:

Group 1: served as the controls, 0.5 ml of 5% Tween-80 in distilled water, p.o

Group 2: glibenclamide 10 mg/kg, p.o

Group 3: CECGL extract 10 mg/kg, p.o

Group 4: CECGL extract 20 mg/kg, p.o

Group 5: CECGL extract 50 mg/kg, p.o

Group 6: CECGF extract 10 mg/kg, p.o

Group 7: CECGF extract 20 mg/kg, p.o

Group 8: CECGF extract 50 mg/kg, p.o

The blood samples from all groups were drawn on the 7, 14, 21, and 27th days after administration of CECGL and CECGF

extracts and were used for estimation of serum glucose levels using a glucose kit.^{30,31} All data collected from the experiment were expressed as the mean \pm standard error (SEM) and analyzed by student *t* test by using Graphpad prism 4.02-version software (USA).^{30,31}

Results

The hypoglycemic effect of CECGL and CECGF extracts oral dose 10, 20 and 50 mg/kg were found to be effective in lowering serum glucose levels in normal rats.

The CECGF at 50 mg/kg exhibited significant ($p < 0.01$) hypoglycemic effects compared to leaves at 3 hrs intervals. The serum glucose levels were proportionately reduced with increase in dose of CECGL and CECGF. It is evident from the results that the CECGF exhibited better hypoglycemic effects compared to CECGL. (Table 1)

For the OGTT, CECGL and CECGF extracts, an oral dose of 50 mg/kg was found to be effective in lowering serum glucose levels. There was a significant ($p < 0.01$) reduction in serum glucose levels at time intervals of 0.5-1.5 hr. (Table 2)

Table 1: Hypoglycemic effect of chloroform extracts of *Calotropis gigantea* leaf and flower in normal rats.

Treatments	Mean blood serum glucose \pm SEM (mg/dl) at the intervals			
	0 hr	1.5 hrs	3 hrs	5 hrs
Control ^a	91.0 \pm 3.0	89.2 \pm 4.9	101.2 \pm 4.5	101.8 \pm 6.2
CECGL ^b 10 mg/kg	85.2 \pm 5.9	69.8 \pm 5.9*	66.8 \pm 6.4*	70.6 \pm 4.0*
CECGL ^b 20 mg/kg	82.8 \pm 3.9	69.9 \pm 6.9*	63.1 \pm 4.9*	63.9 \pm 4.4*
CECGL ^b 50 mg/kg	83.1 \pm 4.2	56.0 \pm 5.4*	51.4 \pm 4.3**	57.5 \pm 3.9*
CECGF ^c 10 mg/kg	82.3 \pm 5.3	57.1 \pm 5.0**	53.8 \pm 4.1*	70.6 \pm 1.6*
CECGF ^c 20 mg/kg	78.9 \pm 4.5	64.9 \pm 4.9*	53.7 \pm 3.4**	61.3 \pm 2.3*
CECGF ^c 50 mg/kg	73.4 \pm 7.5	48.8 \pm 3.2*	39.9 \pm 2.2**	8.2 \pm 2.0*

^a(0.5 ml of 5% Tween 80) CECGL^b: (Chloroform extract of *Calotropis gigantea* leaves); CECGF^c: (Chloroform extract of *Calotropis gigantea* flowers). Values are expressed in terms of the standard error of the mean (SEM) $n=6$ in each group * $p < 0.05$, ** $p < 0.01$. When student *t* test (paired) compared 0 hr with the interval of 1.5, 3 and 5 hr.

Table 2: Effect of chloroform extracts of *Calotropis gigantea* leaf and flower on glucose tolerance in normal rats .

Treatments	Mean blood serum glucose \pm SEM (mg/dl) at the intervals			
	0 hr	0.5 hrs	1.5 hrs	3hrs
Control (glucose 2 g/kg)	78.2 \pm 3.4	153.7 \pm 4.6	144.1 \pm 3.9	122.7 \pm 3.1
Gliben ^a 10 mg/kg + glucose 2 g/kg	75.4 \pm 2.1	116.9 \pm 5.3***	94.56 \pm 3.1***	78.7 \pm 4.0***
CECGL ^b 10 mg/kg + glucose 2 g/kg	86.5 \pm 6.1	132.6 \pm 3.2**	126.0 \pm 3.5**	111.2 \pm 3.8*
CECGL ^b 20 mg/kg + glucose 2 g/kg	82.9 \pm 3.3	127.1 \pm 5.7**	117.4 \pm 4.7**	95.2 \pm 5.2**
CECGL ^b 50 mg/kg + glucose 2 g/kg	80.0 \pm 3.9	125.9 \pm 5.4**	116.4 \pm 7.1**	96.5 \pm 5.3**
CECGF ^c 10 mg/kg + glucose 2 g/kg	83.6 \pm 4.5	132.4 \pm 2.5**	126.7 \pm 2.2**	107.0 \pm 5.5*
CECGF ^c 20 mg/kg + glucose 2 g/kg	76.9 \pm 5.3	131.3 \pm 2.7**	124.8 \pm 2.2**	109.1 \pm 3.8*
CECGF ^c 50 mg/kg + glucose 2 g/kg	77.2 \pm 4.6	129.7 \pm 2.7**	122.0 \pm 1.9***	107.5 \pm 3.4**

^aGlibenclamide; CECGL^b: (Chloroform extract of *Calotropis gigantea* leaves); CECGF^c: (Chloroform extract of *Calotropis gigantea* flowers). Values are expressed in terms of the standard error of the mean (SEM) $n=6$ in each group * $p < 0.05$, ** $p < 0.01$ *** $p < 0.001$, when student *t* test (Unpaired) compared to control.

The anti-diabetic effect of CECGL and CECGF extracts for 27 days of treatment were found to be effective even at an oral dose of 10 mg/kg ($p < 0.001$) in lowering serum glucose levels in STZ-induced diabetic rats. However, the reduction in serum glucose levels was better on the 21st and 27th days of treatment. The results further revealed that the maximum serum glucose suppression occurred on the 27th day after CECGL and CECGF extracts treatment, when compared to the control. Glibenclamide at 10 mg/kg caused a significant ($p < 0.001$) reduction in serum glucose on all days. (Table 3)

Table 3: Effect of chloroform extracts of *Calotropis gigantea* leaf and flower in STZ-induced diabetic rats.

Treatments	Mean blood serum glucose \pm SEM (mg/dl) in days			
	7	14	21	27
Control ^a	346.8 \pm 12.6	351.5 \pm 11.9	346.1 \pm 11.2	352.0 \pm 9.0
Gliben ^b 10 mg/kg	210.6 \pm 8.3***	176.0 \pm 9.4***	192.4 \pm 5.7***	160.0 \pm 8.6***
CECGL ^b 10 mg/kg	262.3 \pm 13.3***	268.9 \pm 18.1**	271.8 \pm 14.1**	275.3 \pm 12.8***
CECGL ^b 20 mg/kg	239.6 \pm 22.6**	263.0 \pm 8.3***	264.8 \pm 6.5***	266.5 \pm 7.4***
CECGL ^b 50 mg/kg	264.3 \pm 13.5**	269.8 \pm 14.5**	262.2 \pm 13.1***	249.1 \pm 18.2***
CECGF ^c 10 mg/kg	239.4 \pm 24.3**	244.3 \pm 24.5**	274.8 \pm 8.0***	270.7 \pm 12.3***
CECGF ^c 20 mg/kg	267.5 \pm 12.7**	271.0 \pm 12.3***	268.1 \pm 8.0***	270.6 \pm 8.2***
CECGF ^c 50 mg/kg	287.1 \pm 7.7**	261.3 \pm 15.2***	271.9 \pm 9.6***	266.5 \pm 12.0***

^aSTZ (55 mg/kg + 0.5ml of 5% Tween 80); ^bGlibenclamide CECGL^b: (Chloroform extract of *Calotropis gigantea* leaves); CECGF^c: (Chloroform extract of *Calotropis gigantea* flowers). Values are expressed in terms of the standard error of the mean (SEM) n=6 in each group *p <0.05, **p <0.01 ***p <0.001, when student t test (Unpaired) compared to control.

Discussion

In order to establish a scientific basis for the utilization CECGL and CECGF extracts in the treatment of diabetes, it was decided to evaluate the hypoglycemic effect, the OGTT in normal and STZ-induced diabetic rats. Earlier reports reveal that STZ-induced diabetic animals may exhibit most of the diabetic complications mediated through oxidative stress.³² Glibenclamide is often used as an insulin stimulant in many studies, and also used as a standard anti-diabetic drug in STZ-induced moderate diabetes to compare the anti-diabetic properties of a variety of hypoglycemic compounds.^{32,33} The findings of our study show that CECGL and CECGF have potent anti-diabetic activity in rats. The preliminary qualitative chemical tests of CECGL and CECGF confirmed the presence of glycosides, alkaloids, flavonoids and tannins by methods,³⁴ supporting the earlier studies on *C. gigantea*.^{10,29} The glycosides may be responsible for observed pharmacological activity.^[33]

Overall, the results show that the CECGL and CECGF possess marked hypoglycemic activity that resulted in improvement of oral glucose tolerance and lowering of the serum glucose levels in STZ-induced diabetic rats. The obtained results provide support for the use of the plant in traditional medicine. On the basis of the above evidence, it may be possible that the presence of flavonol glycosides may be responsible for the observed anti-diabetic activity.^{10,28}

Conclusion

As far as the mechanism of action is concerned, we may speculate that the anti-diabetic activity of the extract may increase the glucose uptake,³³ or may be partly mediated through suppression of the gluconeogenic enzymes, glucose-6-phosphatase.³⁵ However, the precise mechanism(s) and site(s) of activity and active constituent(s) of chloroform extracts of *C. gigantea* leaves and flowers in addition to their toxicological effects have to be determined before concrete recommendation for drug development can be made.

Acknowledgements

The authors declare that they have no conflict of interest to disclose. This research was carried out as a part of doctoral studies by Mr. Nanu Rathod and has received no specific grant from any funding agency in the public, commercial or not-for-profit sectors. The authors would like to thank the Principal at the HSK College of Pharmacy, Bagalkot-Karnataka, India Dr. I. S. Muchandi, for providing all necessary facilities in successfully completing this study.

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