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Demonstration of β -cell regeneration and anti-diabetic activity of *Caesalpinia Pulcherrima* flower extract in alloxan induced diabetic rats

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ABSTRACT

The aim of this study was to evaluate the hypoglycemic activity of the ethanolic extract of *C.pulcherrima* flowers in rat models. The ethanolic extract of *C.pulcherrima* was prepared using 90% ethanol by (1:4) ratio, at 35-40^o C for 6hrs. The extract was distilled and concentrated. A single oral dose of 100, 200 and 300 mg/kg of the extract was evaluated for hypoglycemic activity in alloxan-induced (100 mg/kg) diabetic rats. The blood glucose level was determined at different time intervals i.e. acute and sub-acute. Then the histopathological studies were done for determination of the regeneration of β cells. Dosage of 200 and 300 mg/kg of the extract significantly decreased ($p < 0.05$) blood glucose levels in diabetic rats after 3 h, 1h respectively. There was no significant difference observed at 100 mg/kg. The histopathological study has shown better regeneration of β cells at 200mg/kg, 300mg/kg of extract. The obtained results have shown that the *Caesalpinia pulcherrima* flower ethanolic extract has anti-diabetic activity and detailed investigation is required by using the purified compound from the ethanolic extract of *Caesalpinia pulcherrima*.

Keywords: *Caesalpinia pulcherrima*; Antidiabetic activity; Alloxan-induced diabetic rats

INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia, glycosuria, polyurea and polydipsia induced by insulin deficiency [1] and insulin resistance[2]. Recent estimates indicate that there were 171 million people in world with Diabetes in year 2000 and this may be projected to increase to 366 million by 2030[1].

Diabetes mellitus is treated by using Oral hypoglycemic agents such as Sulphonyl Ureas. Biguanides, Meglitinides and Alpha glucosidase inhibitors [3]. Traditional medicines like herbal drugs in primary form or their extracts have been used by many diabetic patients as they are assumed to be non-toxic in nature[4].

Caesalpinia pulcherrima (L.), Fabaceae, is a leguminous, perennial large shrub or small tree and native of South America. In India, it is cultivated as ornamental plant. Commonly, it is known as Guletura, Gultura (Hindi), Peacock flower, Barbados pride (English), Ratnagandhi (Sanskrit). It is a small tree, 3.7-4.3 m in height. Prickles are sparse on the branches, bark is grey in color. Leaves are abruptly bipinnate, leaflets in 13-20 pairs, 1.3-1.9 cm long. Flowers are red or yellow, fragrant. Flowering season of this plant starts from September to November and fruits from March to April. Traditionally, the flowers, leaves, barks and roots of *C. pulcherrima* have been in clinical use in India since ancient times. Leaves are traditionally used as purgative, tonic, antipyretic and emmenagogue whereas

roots have folkloric use in convulsions, intermittent fevers, lungs and skin diseases [5]. Moreover, the plants of genus *Caesalpinia* are reported to have anticonvulsant activity [6]. Based on traditional and having a verity of Flavonoids [7] which are having very significant antidiabetic activity. So our present study is to investigate the antidiabetic activity of the flowers of *C. pulcherrima* in alloxan induced diabetic rats.

MATERIALS AND METHODS

Drugs and Chemicals:

Glibenclamide and was obtained from Srikrishna Pharmaceuticals Nacharam, Pvt.Ltd, India. Alloxan monohydrate was provided by institution.

Caesalpinia pulcherrima flowers:

The flowers of *Caesalpinia pulcherrima* were collected and authentication was done by botanist Dr. Madhava chetty, Assistant Professor, Department of Botany in S.V. University, Titupathi, and A voucher specimen number (847).

Animals:

Albino wistar rats (150–200 g) of male were purchased from Sainath Agencies, 1-6-197/45/D, Bapujinagar, Musheerabad, (REG.No282/99/CPCSEA), Hyderabad, India and Maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum*. The protocol was approved by the Institutional Animal Ethical committee (IAEC) of Smt.Sarojini Ramulamma College of Pharmacy (R.No: 51/01-CPCSEA/2012/17).

Preparation of the extracts:

The dried flowers were extracted with 95% ethanol by (1:4) ratio, at 35–40⁰ C for 6hrs. The extract was distilled and concentrated [8, 9]. The percentage yield (18.6%) was calculated. The extracts were used for further studies and determination of activity. A preliminary phytochemical screening of the EtOH extract showed presence of flavonoids and steroids.

Phytochemical evaluation[10]:

1. Tests for Flavonoids

A) Shinoda test: Little quatity of extract was taken in a test tube. To this, 5ml 95% ethanol was added followed by the addition of 2ml conc. HCl along the sides of the test tube slowly. Then 0.5g magnesium turnings were added. Appearance of pink colour confirmed the presence of flavonoids.

B) Lead acetate test: Small quantity of residue was taken in a test tube to which lead acetate solution was added. Yellow colour precipitate formed which inferred the presence of flavonoids.

2. Test for Alkaloids

Little quantity of extract was taken in a test tube. To this 2ml of dil.HCL was added. The solution was shaken well and filtered. This filtrate was used to perform the following tests:

A) Dragendroff's reaction: 2 to 3 ml of filtrate was taken in a fresh test tube. To this few drops of dragendroff's reagent was added. Orange brown precipitate was not observed. This inferred the absence of alkaloids.

B) Mayer's test: 2 to 3 ml of filtrate was taken in a test tube followed by the addition of Mayer's reagent. A white precipitate not formed which confirmed the absence of alkaloids.

3. Test for steroids:

Liebermann's reaction: About 1 ml of extract was taken in a fresh clean test tube. To this 1 ml of acetic acid was added. This solution was heated and cooled. Then few drops of conc. sulphuric acid were added along the sides of the test tube. Blue colour was observed. This confirmed the presence of sterols.

4. Test for Terpenoids:

Salkowski reaction: 2ml of extract was taken in a test tube. To this 2ml of chloroform was added. Then 2ml of conc. Sulphuric acid was added along the sides of the test tube slowly and shaken well. Greenish yellow fluorescence was appeared. This confirmed as the presence of terpenoids.

5. Tests for Tannins:

A) Ferric chloride solution test: Little quantity of extract was taken in a test tube. To this, 2ml ethanol was added and mixed well followed by the addition of 1ml of 5% ferric chloride reagent. Deep blue colour was not observed which inferred the absence of tannins.

B) Lead acetate test: 2ml of extract was taken in a test tube followed by the addition of alcohol and shaken well. To this 2 ml lead acetate was added, no precipitate formed which inferred the absence of tannins.

6. Test for carbohydrates:

Molisch's test: To 2-3 ml of extract few drops of molisch's reagent (alpha naphthol solution in alcohol) was added. The test tube was shaken well and concentrated sulphuric acid was added along the sides of the test tube. Formation of violet ring at the junction of two liquids was observed. This inferred the presence of carbohydrates.

Hypoglycemic activity:**Induction of diabetes:**

Alloxan monohydrate was first weighed individually for each animal according to its weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 100 mg/kg b. wt. intraperitoneally [11, 12]. After 1 hr of alloxan administration, the animals were given feed *ad libitum* and 5% dextrose solution was also given in feeding bottle for a day to overcome the early hypoglycemic phase [13, 14]. The animals were kept under observation, and after 48 hr, blood glucose was measured. One group served as a control which received vehicle alone. The diabetic rats (glucose level > 150 mg/dl) were separated and divided into different groups for experimental study [15].

Experimental design:**Acute treatment and subacute treatment:**

Four groups of animals containing six rats in each group were divided as follows. Group I: Normal control (saline), Group II: Diabetic control (saline), Group III: Standard (Glibenclamide 10mg/kg) Group IV, V and VI were treated orally with 100mg/kg, 200mg/kg and 300mg/kg/day of ethanolic extract of *C.pulcherrima* flower, respectively. Treatment continued for seven days.

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated at 0, 1, 3 and 5 h and 1, 3, 5, and 7 days [13,14]. Blood glucose levels were determined by GOD-POD method [16, 17, and 18].

Histopathological studies:

The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and Sections of 5 μ thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination [19,20,21].

Statistical analysis:

All the values of the experimental results were expressed as mean \pm SEM and analyzed by one way ANOVA followed by "Dunnett's Test"

RESULTS

The hypoglycemic effect of the ethanolic extract of *Caesalpinia pulcherrima* flowers on the fasting blood sugar levels of diabetic rats is shown in the Tables 1 and 2 and histopathological in figure 1. After a single dose of extract the doses of 200 mg/kg and 300mg/kg showed a significant reduction in the blood sugar level after 3 h, and 1 h respectively, in the subacute study the test-1 and test-2 started significant reduction in blood glucose level from 3rd day, and test-3 started from 2nd day of the treatment. At the end of the study the extract at dose of 200 mg/kg and

300 mg/kg showed a significant ($p < 0.001$) reduction in the blood glucose level comparable with that of glibenclamide (10 mg/kg) treated group.

From the histopathological studies it was suggested that the regeneration of β cells was observed in all test groups and standard group but better regeneration was observed in test-2 and test-3 when compared to diabetic control group i.e. equal to that of standard drug treated group. The results showed that the better regeneration of β cells is observed in test-3 (300mg/kg) group and standard drug treated group when compared to diabetic control group.

Table 1 Effect of *C. pulcherrima* flower extract on alloxan-induced diabetic rats after single dose

Treatment	Blood glucose level (mg/dl)			
	0 h	1 h	3 h	5 h
Normal control	87.50 \pm 2.802	87.33 \pm 2.578	86.67 \pm 2.539	86.33 \pm 2.578
Diabetic control	189.80 \pm 2.638	189.90 \pm 2.028	192.80 \pm 2.688	195.00 \pm 2.646
Standard	166 \pm 2.633	161.70 \pm 2.77	150.50 \pm 2.643**	134.50 \pm 2.790***
Test – 1	177.30 \pm 4.470	175.30 \pm 4.529	169.80 \pm 4.468	161.80 \pm 3.341
Test – 2	178.00 \pm 5.183	177.00 \pm 4.155	167.20 \pm 3.945	161.20 \pm 4.55*
Test-3	181.20 \pm 3.016	176.50 \pm 2.975	157.80 \pm 2.822***	147.80 \pm 2.664***

Values are mean \pm SEM; N=6. *P value 0.01, **p 0.02, ***p < 0.001 vs. diabetic control.

Histopathology of rats:

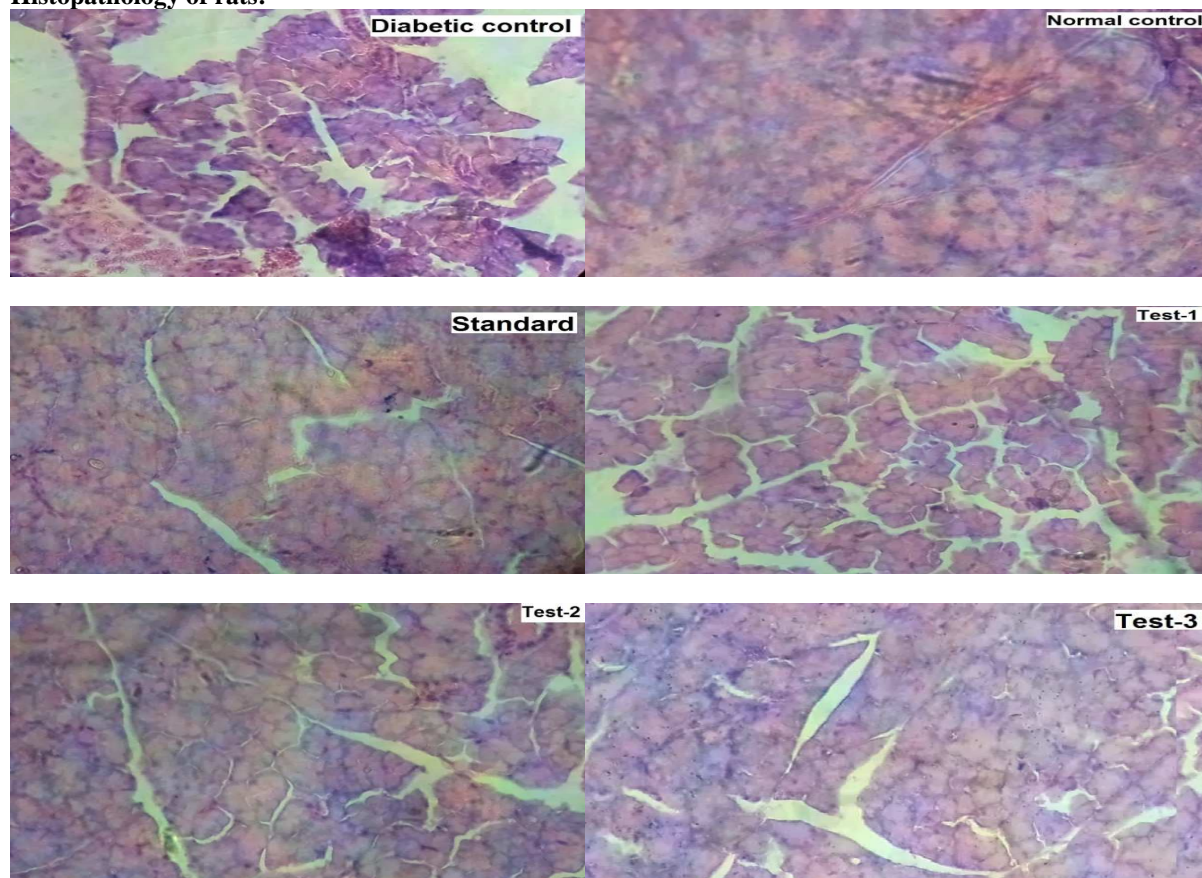


Figure 1: Regeneration of β -cells in standard and test groups after subacute treatment.

Table 2 Effect of *C. pulcherrima* flower extract on alloxan-induced diabetic rats after sub-acute treatment

Treatment	Blood glucose level (mg/dl)				
	0 day	1 day	3 day	5 day	7 day
Normal control	87.50±2.80	86.94±2.669	87.17±2.522	87.17±2.688	86.67±2.789
Diabetic control	189.80±2.638	192.40±2.347	216.70±3.556	235.20±2.330	258.20±3.240
Standard	166±2.633	148.90±2.724***	135.20±2.442***	114.20±2.272***	93.00±3.044***
Test- 1	177.30±4.470	169±4.091	169.30±4.602*	167.20±4.285**	163±4.4.604***
Test- 2	178.20±5.183	168.40±4.143	161±4.4.344*	155.20±4.676**	146.80±4.512***
Test- 3	181.20±3.016	160.70±2.812**	148.80±2.414***	131.30±2.186***	110±2.503***

Values are mean±SEM; N=6. *P value 0.01, **p 0.02, ***p < 0.001vs. Diabetic control.

DISCUSSION

Diabetes mellitus is a common heterogeneous metabolic syndrome, is prevalent throughout the world and has been project to become of the world's main disablers and killers within the next 25 years. Blood glucose, urine sugar and body weight have been commonly measured to monitor the glycemic control mechanism.

Caesalpinia pulcherrima (L.) is widely used in india since ancient times. Leaves are traditionally used as purgative, tonic, antipyretic and emmenagogue whereas roots have folkloric use in convulsions, intermittent fevers, lungs and skin diseases. Moreover, the plants of genus *Caesalpinia* are reported to have anticonvulsant activity. Based on traditional and having a verity of Flavonoids which are having very significant antidiabetic activity. So our present study is to investigate the antidiabetic activity of the flowers of *C. pulcherrima* in alloxan induced diabetic rats.

After a single dose of extract the doses of 200 mg/kg and 300mg/kg showed a significant reduction in the blood sugar level in acute subacute study.

From the histopathological studies it was suggested that the regeneration of β cells following destruction by alloxan might be the primary cause for the antidiabetic activity of the extracts. There is no destruction of β cells in normal control group, complete damage of β cells was observed in diabetic control group, and regeneration beta cells was observed in all test groups and standard group but better regeneration was observed in test-2 and test-3 when compared to diabetic control group i.e. equal to that of standard drug treated group. The results showed that the better regeneration of β cells is observed in test-3 (300mg/kg) group and standard drug treated group when compared to diabetic control group.

CONCLUSION

From the above discussion it concludes that the ethanolic extracts of the *Caesalpinia pulcherrima* flower (200mg/kg and 300mg/kg) exhibited significant antidiabetic activity against alloxan induced hyperglycemia. These extracts also showed improvement of regeneration of β cells of pancreas and so might be of value in diabetes treatment. Further investigation is in necessary to determine the exact phytoconstituents (s) responsible for antidiabetic effect and mechanism of action.

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