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EVALUATION OF ANTIDIABETIC ACTIVITY OF ETHANOLIC EXTRACT OF STEMS OF *HIBISCUS PLATANIFOLIUS* IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

The hypoglycemic effect of ethanolic extract of stems of *Hibiscus plantanifolius* was evaluated in alloxan - induced diabetic rats. Oral administration of extract (250, 500 /kg bw) for 28 days resulted in a significant reduction in blood glucose level. The effect was compared with 0.5gm/kg of glibenclamide (i.p). Alloxan induced hyperglycemia rat models were used for the evaluation of anti-diabetic activity. The effect of ethanolic extract of stems of *Hibiscus plantanifolius* of normal glycemic, Alloxan induced hyperglycemic activity were showed in dose dependant manner. The present study was design to evaluate ethanolic extract of stems of *Hibiscus plantanifolius 250* mg/kg and 500 mg/kg p.o dose against Diabetic in rats. It was evaluated by Physical parameter like Body weight, and Biochemical Estimations by Serum parameters: glucose, triglycerides, cholesterol, HDL, LDL levels.

KEYWORDS: Antidiabetic activity, alloxan, Glibenclamide, *Hibiscus plantanifolius.*

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism As the disease progresses tissue or vascular damage ensures leading to severe diabetic complications such as retinopathy, neuropathy, nephropathy, cardiovascular complications and ulceration Thus, diabetes covers a wide range of heterogeneous diseases.[1] The effective control of blood glucose is the key in preventing or reversing diabetic complications and improving the quality of life for both type I and type II diabetic patients. Although different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, none is offering complete glycemic control.[2]

Traditional plant medicines are used throughout the world for a range of diabetic presentations. Therefore, investigation on such agents from traditional medicinal plants has become more important. India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes.[3]

Hibiscus platanifolius of the family Malvaceae has been widely cultivated in countries all over the world including temperate to tropical areas. Different parts of the plant are used as hyperlipidemic, hypertension, liver disorders and as antidotes to poisoning chemicals. In the present review focused evaluation of antidiabetic activity of *Hibiscus plantanifolius.*

MATERIALS AND METHODS

Plant material

Hibiscus platanifolius were collected from the Tirumala hills of Chittor district. Its botanical identity was authenticated by prof. k.Madavschetty, Department of Botany, Sri Ventateswara University, Tirupati, A.P. These were bark was separated and prepare coarsely powdered for preparation of extraction.

Preparation of extracts

Each 350 g bark powder of *Hibiscus platanifolius was* collected & extracted using 90% ethanol for *Hibiscus platanifolius, on a reflux water bath for 3 hr.* The cycle was repeated for three times. The extract was concentrated on rotary flash evaporator to semi solid consistency .To it 1-2 drops of chloroform was added and stored at 8º C in screwed glass vials.

Experimental animals

Male Wister albino rats weighing 150-200 g were used in the present study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animals were given standard rat pellets (Pranav Argo's ltd.) and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal

Ethical Committee of Creative Educational Society College of Pharmacy (1305/ac/09/CPCSEA).

Chemicals and Reagents
Normal Saline, Ethanol, Alloxan, Glucose kit, Triglyceride kit, HDL kit, LDL kit, Cholesterol kit (estimation kits were procured from Kamineni Life Sciences Pvt. Ltd. India).

Equipments: Semi Auto analyzer (MISPEL).

Phytochemical screening
The ethanolic extract of stems of *Hibiscus platanifolius was* subjected to various qualitative chemical tests to detect the chemical constituents present in it in the following manner.[4, 5, 6, 7]

Test for Alkaloids
Dragendroff's Test
0.5 gm of the crude dried powder was warmed with 10 ml. of 2% sulphuric acid for 2 minutes and filtered. The solution was used for the tests. 1 ml. portion was treated with a few drops of dragendroff's reagent, brownish red colour precipitate was observed showing the presence of alkaloids.

Wagners test
To 1 ml of the solution, 1 ml. of Wagner s reagent (iodine in potassium iodide) was added. Yellow colour precipitate was observed indicating that alkaloids are present.

Test for Saponins
Salkowski test
A small amount of extract was taken in a test tube containing 1 ml. chloroform and added 5 to 6 drops of conc. sulphuric acid. Blood red color in lower layer was formed indicating the presence of steroidal saponins.

Antimony trichloride test
A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added 3 ml. of antimonytrichloride followed by heating. Color change was observed (purple to violate color) indicating the presence of steroidal saponins.

Test for Tannins
About 0.5 Gms. of the extract was stirred with about 10 ml. of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml. of the extract in methanol. Blue-green precipitate was formed indicating the presence of tannins.

Test for Flavonoids
Shinoda test
A small amount of the extract was taken in a test tube containing 1 ml. of ethanol and added a mixture containing magnesium ribbon and concentrated hydrochloric acid. Red color was observed indicating the presence of flavonoids.

Lead acetate test
A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added 1 ml. of lead acetate solution.Colored precipitate was formed indicating that flavonoids are present.

Test for Steroids
Libermann-burchard test
A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added4 to 5 drops of acetic anhydride and 4 to 5 drops of conc sulphuric acid. Blue color was observed. It indicates that steroids are present.
A small amount of extract was taken in a test tube containing 1 ml. of chloroform and added 3 ml. of antimonytrichloride followed by heating. Color change was observed (purple to violate color). It indicates that steroids are present.

Test for Anthracene glycosides
A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added 0.5 ml. of potassium hydroxide solution. Violate color was observed indicating that anthracene glycosides are present.

Test for Amino acids
Ninhydrin test
A small amount of extract was taken in a test tube containing 1 ml. of ethanol and added ninhydrin reagent, followed by heating. Purple color was observed, indicating that amino acids are present.

Acute toxicity test
The acute toxicity of ethanolic extract of *Hibiscus platanifolius* was determined in mice according to the method of Hilaly et al. with slight modifications. Rats fasted for 12 h were randomly divided into groups of six rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered to the rats in each of the groups by means of bulbed steel needle. All the animals were then allowed free access to food and water and observed over a period of 48 h for signs of acute toxicity. The number of deaths within this period was recorded. . Based on the results of preliminary toxicity test, the doses of 250 and 500 mg/kg body weight (according to OECD Guideline 420) of ethanolic extract of *Hibiscus platanifolius* were chosen for further experiments.[8]

Pharmacological studies
Oral Glucose tolerance test (OGTT)
Rats were fasted overnight and divided into five groups with 6 animals in each group. Group-I received distilled water, to serve as control. Group-II animals were treated with Glimipramide (0.5 mg / kg) to serve as standard. Group-III animals were treated with *Hibiscus platanifolius* extract (500mg/kg, B.wt), The groups control, standard and test were treated with drugs 30 minutes prior to the glucose load (2.5 g/kg). Blood samples were collected at 15, 30, 45, 60, 75, 90 and 120

min after glucose loading. Serum was separated and glucose levels were measured immediately.[9]

Anti diabetic study

In the present study, diabetes was induced by single intraperitoneal injection of alloxan (125mg/kg). The alloxan was freshly prepared by dissolving 125mg of alloxan in 1ml of normal saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia.[10]

72 hours after injection of alloxan, fasting plasma blood glucose was estimated. Animals with plasma glucose of > 140 mg/dl were included in groups II-V. The rats were divided into five groups consisting of five rats in each group; the animals were treated for 28 days (Table-1).

Table -1: Treatment schedule for antidiabetic activity

Group No.	Treatment	Purpose
I	No treatment	To serve as normal control
II	Alloxan + Distilled water (125mg/kg i.p)	To serve as disease control
III	Glimipramide (0.5 mg/kg.)	To serve as standard
IV	*Ethanolic extract of Hibiscus platanifolius* (250mg/kg,)	To study the antidiabetic effect of *Hibiscus platanifolius*
V	*Ethanolic extract of Hibiscus platanifolius* (500mg/kg,)	To study the Antidiabetic effect of *Hibiscus platanifolius*

Collection of blood sample

The blood samples were drawn on 7th, 14th, 21st and 28 th day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged (3,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, cholesterol, HDL-cholesterol.

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was performed by One-way analysis of variance (ANOVA) test for multiple comparisons followed by Turkey-Kramer test. Statistical significance was set accordingly.

Table -2: phytochemicl constituents.

NAME OF COMPONENT	RESULT
Test for phenols	+
Flavanoids	+
Saponins	+
Steroids	+
Glycosides	+
Terpinoids	+
Alkalods	+

Acute toxicity studies

The ethanol extract of *Hibiscus platanifolius* did not show any mortality and toxic manifestations up to the dose of 3200 mg/kg. b.w. Further dosing was not performed to estimate the LD_{50} (lethal dose) value. According to the OECD guidelines for the acute toxicity, an LD_{50} dose of 2000 mg/kg and above is cat egorized as unclassified and hence the drug is found to be safe. Based on the acute toxicity studies, the dose 200 mg/kg of the fractions has been selected as the therapeutic dose.

1. Effect of *Hibiscus platanifolius* on glucose tolerance in normal fasted rats.

OGTT test was studied by administration of glucose (5 mg/kg, p.o) to control, standard and test. Control (G-II) animals, a significant increase in blood glucose levels were noticed after 60 min which was followed by a reduction after 120 min. (Table-3)

Treatment with standard drug glimipramide (group-III), blood glucose raised at 30 min followed by subsequent fall up to 120 min. It was observed from present study that administration *Hibiscus platanifolius* extracts increased the glucose levels were seen after 30 min and hypoglycemia effect was observed only after 120 min.

RESULTS

Phytochemical Screening

The preliminary phytochemical studies RDE received that presence of Phenols, Flavanoids, Steroids, Glycosides, terpinoids and absence of alkaloids as shown table -2.

2. Effect of *Hibiscus platanifolius* on serum glucose levels

In animals treated with alloxan (G-I) (125 mg/kg i.p) a significant increase in the serum glucose levels was observed on the 7th, 14 th, 21st and 28th day, when compared to the normal group (G-I).

Group-III treated with standard drug (glimepramide– 0.5 mg/kg p.o) showed a significant decrease in serum glucose levels on 7th, 14th, 21st and 28th day, when compared to the diabetic control group (G-II).

On administration of *Hibiscus platanifolius* extract groups (G-IV and G-V), the blood glucose levels were decreased on7th, and 14th, 21st and 28th day, when compared to the control group (G-II) (Table-4).

3. Effect of *Ethanolic extract of Hibiscus platanifolius* on serum triglyceride levels

Group –II animals receiving alloxan showed a significant increase in triglyceride levels on 14th ,21st and 28th day when compared to the normal group (G-I).Rats treated with standard drug (G-III) had significantly lowered triglyceride level on 14 Th, 21st and 28th day when compared to the control group (G-II). On administration of *Hibiscus platanifolius* extract groups (G-IV and G-V), the blood triglyceride levels were decreased on7th, 14th, 21st and 28th day, when compared to the control group (G-II)

4. Effect of *Hibiscus platanifolius* on serum cholesterol

The biochemical parameter, serum cholesterol has shown significant increase in alloxan induced group (G-II) when compared with the normal group (G-I). A significant decrease in the levels of serum cholesterol was observed from 14th day onwards on administration of glimeprmide (G-III), when compared with the control group (G-II). On administration of *Hibiscus platanifolius* extract groups (G-IV and G-V), the blood serum cholesterol levels were decreased on 7th, 14th, 21st and 28th day, when compared to the control group (G-II)

5. Effect of *Hibiscus platanifolius* on serum HDL level

The rats induced with alloxan(G-II) a significant decrease in HDL levels was observed on 14th ,21st and 28th day, when compared to the normal group(G-I).

Group-III, receiving standard drug (glimepramide-0.1 mg/kg p.o) showed a significant increase in HDL levels on 14th, 21st and 28th day, when compared to the control group (G-II). Administration of *Hibiscus platanifolius* extract groups (G-IV and G-V), have shown a significant increase in HDL levels on 7th ,21st and 28th day, when compared to control group (G-II).(Table-4)

6. Effect of *Hibiscus platanifolius* on serum LDL level

The rats induced with alloxan(G-II) a significant increase in LDL levels was observed on 14th ,21st and 28th day, when compared to the normal group(G-I).

Group-III, receiving standard drug (glimepramide-0.1 mg/kg p.o) showed a significant decrease in LDL levels on 14th, 21st and 28th day.

7. Effect of *Hibiscus platanifolius* on body weight

The rats induced with alloxan (G-II) a significant decrease in body weight was observed on 7th, 14th ,21st and 28th day, when compared to the normal group(G-I). Group-III, receiving standard drug (Glimiperide drug(1 mg/kg) , G-IV and G-V showed a significant increase in body weight on 14th ,21st and 28th day , when compared to the control group (G-II).

Table 3: Effect of *Hibiscus platanifolius* on glucose tolerance in normal fasted

Group	Treatment	Serum glucose (mg/dl) (mean±SEM)				
		Time after glucose administration in minutes				
		0	30	60	90	120
I	Control	69.62±4.22	103.51±4.44	139.61±4.52	159.17±4.26	116.27±5.25
II	Standard	64.64±6.57	72.31±6.22[a]	67.59±5.69[a]	64.19±6.39[a]	64.12±5.86
III	***Hibiscus platanifolius250mg/kg***	66.84±4.19	86.19±4.24[a]	93.33±5.61[a]	96.32±4.21[a]	68.43±5.18[a]
IV	***Hibiscus platanifolius 500mg/kg***	67.39±4.93	85.26±5.15[a]	90.19±5.20[a]	94.21±5.61[a]	65.6±4.34[a]

a = $p < 0.001$, when compared to control. (G-I)

Table-4: Effect of *Hibiscus platanifolius* on 28th day in diabetic rats.

Group	Treatment	Biochemical parameter levels (Mean±SEM)				
		glucose (mg/dl)	Triglyceride (mg /dl)	cholesterol (mg /dl	HDL (mg /dl)	LDL (mg /dl)
I	Normal	82.13±5.19	159.37±13.22	75.43±6.67	61.99±8.32	63.47± 6.45
II	Control	239.12±15.26[a]	253.91±19.33[b]	158.68±14.18[a]	31.08± 3.91[b]	138.04±10.45[a]
III	Standard	85.16±7.58[b]	155.99±14.43[d]	79.14±7.73[b]	69.08±7.31[d]	71.12± 5.25[c]
IV	***Hibiscus platanifolius 250mg/kg***	109.11±9.45[b]	180.15±15.41[d]	112.00±11.34[b]	55.30±6.64[d]	89.37±7.45[c]
V	***Hibiscus platanifolius 500mg/kg***	90.90±7.18[b]	158.22±11.13[d]	85.61±8.41[b]	67.18± 8.51[c]	79.76± 6.18[c]

a =$p < 0.05$, when compared to normal. (G-I)
b =$p < 0.001$, when compared to normal. (G-I)
c =$p < 0.01$, when compared to control (G-II)
d =$p< 0.001$, when compared to control. (G-II)

Table-5: Effect of *Hibiscus platanifolius* on body weight levels in diabetic rats

Group	Treatment	Body weight (grams) (Mean ± SEM) on				
		0th day	7th day	14th day	21st day	28th day
I	Normal	178±1.76	181.8±0.58	185.4±0.92	187.4± 0.77	190.6±1.66
II	Control	175.2±1.80	170.8±0.56[a]	159.2±1.68[a]	150.4± 1.43[a]	138.4± 1.28[a]
III	Standard	177.4±2.67	173.6±0.50[c]	179.2± 0.37[b]	184.2±1.06[b]	188.4±1.40[b]
IV	***Hibiscus platanifolius 200mg/kg***	181.2±0.96	175.0±0.70	162.6±0.89	167±0.54[c]	168.8±0.58[c]
V	***Hibiscus platanifolius 500mg/kg***	180.5±0.92	174.8±0.86[c]	178.8± 0.96[b]	182.6±1.20[b]	188.6± 1.03[b]

a =p < 0.001, when compared to normal. (G-I)
b =p < 0.01, when compared to control. (G-II)
c =p < 0.001, when compared to control. (G-II)

DISCUSSION

Diabetes mellitus is one of the leading causes of death, illness and economic loss all over the world. Insulin-dependent (Type I, IDDM) diabetes is characterized by juvenile onset and by absolute insulin deficiency. Non-insulin-dependent (Type II, NIDDM) diabetes is characterized by mature onset, by varying basal insulin levels and a frequent association with obesity.

Alloxan is a cyclic urea compound, which induces permanent diabetes. It is a highly reactive molecule, which produces free radical damage to beta islet cells & causes cell death. When islets are exposed in vitro to alloxan, it exhibits exceptional beta cell specificity, the other islets cells remaining largely unaffected by both its inhibitory and cytotoxic effects. Alloxan is a specific toxic substance that destroys the β cells provoking a state of primary deficiency of insulin without affecting other islet types (Dunn et al., 1943; Goldener et al., 1964). The damage occurs in nerves; hence, alloxan was selected to induce diabetes in the present study. Almost all the flavonoids having potential for antidiabetic activity but they are limited in usage on account of deprived solubility and bioavailability.[11, 12, 13]

A number of plants have been reported to possess hypoglycemic effects and the possible mechanism suggested for such hypoglycemic actions could be through an increased insulin secretion from β-cells of islets of Langerhans or its release from bound insulin or such hypoglycemic effects of plant extracts could also be because of their insulin-like actions.(Twaij and Badr., 1988; Kasiviswanath et al., 2005) Similar mechanisms may be considered responsible for the hypoglycemic action shown by of *Hibiscus platanifolius* in diabetic rats.

Different chemical agents are capable of producing the alterations related to the diabetic condition.[14] Alloxan became the first diabetogenic chemical agent when Dunn and Letchie accidentally produced islet-cell necrosis in rabbits while researching the nephrotoxicity of uric acid derivatives. Alloxan is a specific toxic substance that destroys the β cells provoking a state of primary deficiency of insulin without affecting other islet types.[15] Hence, alloxan was selected to induce diabetes in the present study.

In the present study, preliminary phytochemical screening of extracts showed the presence of flavonoids, steroids, terpenoids and alkaloids. Antidiabetic activity of extracts may be due to its high content of flavonoids and steroids.[16]

Flavanoids usually reduction of aldose reductase, regeneration of pancreatic cells to enhance the insulin releases. Literature survey revealed flavonoids and phenols are effective antihyperglycemic agents which can regenerate the damaged β cells in alloxan induced diabetic rats and produce analgesic action and also plant contain rich amount steroids. Steroids also showed analgesic activity, in the present study.

Similar mechanisms may be considered responsible for the hypoglycemic action shown by of *Hibiscus platanifolius* in diabetic rats.[17, 18, 19]

CONCLUSION

From this study, we can state that the ethanolic extract of *Hibiscus platanifolius* has beneficial effects on blood glucose levels as well as improving the hypoglycemic action. These observations clearly indicate the potential of *Hibiscus platanifolius* to reduce gluconeogenesis. Thus, of *Hibiscus platanifolius* in diabetic rats reduced blood glucose levels and increased glycogenesis and glycolysis, reduced gluconeogenesis and brought the glucose metabolism towards normal levels. Moreover, the effect of high dose of plant extract in diabetic rats is found to be similar to that of glimepramide.

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