ORIGINAL ARTICLE

Hypoglycemic activity of *Cassia javanica* Linn. in normal and streptozotocin-induced diabetic rats

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ABSTRACT

In present work, one of the ornamentals and medicinally less known plant *Cassia javanica* has been explored for hypoglycemic potential. It aimed to check the hypoglycemic effect of *C. javanica* leaves on normal and streptozotocin (STZ)-induced diabetic rats by acute and sub-acute studies. Prior to the hypoglycemic study, acute oral toxicity testing of drug was performed. Later, the effects of single and multiple doses of test drug were studied using various parameters. Dried powdered leaf material was used as an oral drug. The preliminary phytochemistry of drug was done by standard qualitative tests. Diabetes was induced in rats by single intraperitoneal injection of STZ. Single and multiple doses of test drug (0.5 g/kg body weight/day) were given to normal and diabetic rats. The parameters studied were blood glucose, serum cholesterol, serum triglycerides, and serum proteins. The results of test drug were compared with standard hypoglycemic drug-glibenclamide (0.01 g/kg/day). Statistical analysis was done by 'Student's '*t*' test' and one way ANOVA test. In preliminary phytochemistry, antidiabetic compounds were detected. Unlike acute, subacute treatment of test drug showed highly significant reduction (37.62%) in blood glucose level of diabetic rats in ten days. This effect was considerably good in comparison with standard drug (63.51%). The test drug and standard drug exhibited insignificant change in the abnormal levels of serum metabolites of diabetic rats. Preclinically, *C. javanica* was proved to be effective hypoglycemic agent.

Key words: Acute toxicity, *Cassia javanica* leaves, hypoglycemic, streptozotocin

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder that is mainly characterized by abnormally elevated blood glucose level. It shows altered metabolism of carbohydrates, lipids, and proteins with an increased risk of vascular and renal diseases.[1,2] Despite harmful side effects, insulin and synthetic oral hypoglycemic agents are widely used in management of Diabetes mellitus.[3] Frequently, herbal remedies are also preferred as they are safe for long-term use, easily accessible, and cost effective. There are many antidiabetic herbs recommended in traditional medicaments but still there is a worldwide quest for an ideal drug due to complex nature of the disease. Hence, it is essential to screen and evaluate new unexplored plants for antidiabetic potential for the development of complete drug.

Cassia javanica Linn. (English – Java Cassia) is a beautiful garden tree that belongs to family Leguminosae. It is cultivated throughout India for beautiful pink blossoms.[4,5] Previous literature provides meagre information about therapeutic uses of the plant. Bark of *C. javanica* is used as one of the ingredients in antidiabetic ayurvedic formulation.[6] Leaves are proved to be active against *Herpes simplex* infection.[7] Leaves are reported to contain variety of secondary metabolites, such as, flavones, sterols, several hydrocarbons, anthraquinones, glycosides, etc.[8,9] Among these flavones, glycosides and sterols are considered to

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be antidiabetic compounds.[10,11] The presence of these antidiabetic phytochemicals of *C. javanica* leaves may give desired pharmacological action. As there are no scientific data available regarding antidiabetic effects of leaves, it felt relevant to assess bioactivity of leaves of *C. javanica*.

MATERIALS AND METHODS

Plant Material

The samples of leaves of *C. javanica* were procured from adjoining regions of Mumbai and Bangalore. The mature leaves were obtained during their flowering season of May to July. The botanical identity was confirmed using the standard herbaria at Botanical Survey of India (BSI), Pune (Accession No. BSI Kumavat-1). Leaf samples were subjected to artificial drying at 40°C and ground to form powder. The powdered drug samples were moderately coarse as they were seivable through mesh no. 710 with 0.710 mm size of aperture.[12,13] It was stored in closed, airtight containers with silica bags.

Preliminary Phytochemical Analysis of Drug

5 g of powdered drug was macerated with 100 ml of water, ethanol and chloroform separately. The solutions were subjected to frequent shaking for 18 hours and then were allow standing for 6 hours. The filtrates obtained from respective solvents were concentrated and subjected to different tests for the identification of phytochemical constituents.[14,15]

Animals

Laboratory bred male Wistar albino adult rats weighing 200 to 250 g were used for the studies. All the animals were procured from Haffkine Bio-Pharmaceutical Corporation, Mumbai. The animals were housed in standard environmental conditions of temperature (21 ± 2°C), humidity (55 ± 10%), and a 12 hour light-dark cycle. They were supplied with commercial pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethic Committee of R.J. College, Mumbai (Registration No. 525/02/a/CPCSEA, Approval No. 8/5-8-2010).

Chemicals

The different chemicals used during the study were streptozotocin (STZ) (Sisco Pharmaceutical Limited, Mumbai) and glibenclamide (Aventis Pharma Limited, Verna, Goa). Glucometer with Blood gluco-strips (SugarScan Thyrocare Technology Limited, Navi Mumbai), Diagnostic kits of total cholesterol, triglycerides, and total proteins (Agappe Diagnostics Ltd., Pattimattom, Ernakulum), and rest all other reagents and chemicals were of analytical grade.

Acute Toxicity Study

Acute toxicity study was carried out as per the procedure given in OECD Guideline No. 420.[16] The male Wistar albino rats (200 to 250 g) was used in the study. After sighting study, the drug of *C. javanica* at the dose of 2 g/kg body weight was given to five animals. The animals were continuously observed for 14 days for mortality and general behavior. No death was observed till the end of the study. The drug was considered safe up to the dose of 2 g/kg body weight. From the results, test drug dose of 0.5 g/kg body weight was chosen for the efficacy studies.

Induction of Diabetes by Streptozotocin

Rats were fasted for 16 hours and then a single intraperitoneal injection of 0.05 g/kg body weight STZ in 0.1M Citrate buffer (pH 4.5) was given to them. The fasting blood glucose levels were checked after three days. The rats with stable fasting blood glucose level above 250 mg/dl were used for the acute and sub-acute efficacy studies. After induction of diabetes, all the animals were kept in laboratory on normal diet.[17-19]

Acute Study on Normal and Streptozotocin induced Diabetic Rats

To determine the hypoglycemic activity of the drug, normoglycemic rats were fasted for 18 hours. They were divided into two groups of six rats each. Group I served as Normal Control and received orally 2% gum acacia (vehicle). Group II animals were fed with test drug of *C. javanica* at oral dose of 0.5 g/kg body weight in vehicle. Similarly, for testing drug activity on diabetic rats, 18 hours fasted animals were distributed into three groups, each containing six rats. Group III served as Diabetic Control and was given 2% gum acacia vehicle. Group IV was fed test drug of *C. javanica* at the dose of 0.5 g/kg body weight in vehicle. Dosing of 0.01 g/kg body weight of Standard oral hypoglycemic agent Glibenclamide was done for Group V. The blood was withdrawn by tail vein puncturing. The samples of blood were obtained at zero, second, third, and fourth hour of the treatment. The blood glucose levels were determined using Glucometer.[11,20,21]

Sub-acute Study on Diabetic Rats

In sub-acute method, three groups of diabetic rats viz., Group I, II, and III were made. Normal Control was kept as Group IV. Diabetic Control Group I and Normal Control Group IV received 2% gum acacia vehicle only. Group II was given oral test drug (0.5 g/kg body weight). Group III was given 0.01 g/kg body weight Glibenclamide orally. In a day, single dosing was done in the morning and the treatment continued till tenth day. During the study, fasting blood glucose levels were checked on zero, second, fourth, sixth, eighth, and tenth day of experiment. The biochemical parameters such as total cholesterol, triglycerides, and total proteins were estimated from serum samples on zero and tenth day. The samples of serum obtained from blood withdrawn from retro-orbital plexus. Commercial kits of Agappe Diagnostics were used for biochemical estimations.[21-23]

Statistical Analysis

The values of all parameters are expressed as Mean±Standard Error in tables. The data were statistically analyzed by

'Student's '*t*' test' and one-way ANOVA test. *P* values < 0.05 and < 0.01 were considered to be significant.

RESULTS

The preliminary phytochemical analysis of *C. javanica* leaves revealed the presence of reducing sugars, proteins, alkaloids, tannins, glycosides, sterols, flavonoids, and saponins. As some of antidiabetic biomolecules (glycosides, flavonoids, and sterols) were detected in phytochemical screening, the pharmacological investigation of the said drug has been done for the first time.

Initially, in acute oral toxicity study, *C. javanica* leaves did not show any toxicity sign in rats at the dose of 2 g/kg body weight. The animal behavior was found to be unchanged. Thus, it ensured the safe nature of an oral drug. Further, in order to ascertain the usefulness of drug against Diabetes mellitus, acute and sub-acute modes of treatment were followed.

Acute treatment involved administration of the single dose (0.5 g/kg body weight) of test drug. It failed to show hypoglycemic effect till fourth hour of drug administration. The drug produced insignificant reduction in blood glucose levels of normal (Group II) and diabetic rats (Group IV) compared with Normal Control (Group I) and Diabetic Control (Group III), respectively. At the same time, Standard drug (Group V) exhibited quite significant (P<0.05) results with 20.73% reduction in comparison with diabetic control [Tables 1 and 2].

In contrast, sub-acute treatment indicated excellent hypoglycemic activity of the drug. In ten days period, test drug (0.5 g/kg body weight/day) started significant action (P<0.05) from eighth day in Group II. Due to consistent decrease in glucose concentration, total reduction of 37.62% was achieved at the end of treatment. This result was highly significant (P<0.01) when compared with Diabetic Control (Group I). Similarly, Standard drug (Group III) brought down the glucose level significantly (P<0.01) from second day, with total reduction of 63.21% in ten days treatment. Although test drug induced much delayed response, quite considerable total reduction in blood glucose was achieved in less time. It showed more than half % reduction as compared with the % reduction of blood glucose exhibited by standard drug [Table 3].

As a part of sub-acute study, the action of drug was also analyzed on serum metabolites. In diabetic rats, the levels of total cholesterol and triglycerides were abnormally elevated and total proteins level was noticeably reduced as compared with Normal Control (Group IV). Biochemical assays were done on zero day and tenth day of treatment. In Diabetic Control (Group I), there was slight increase in levels of total cholesterol and triglycerides, whereas total protein level was lowered in ten days period. Almost similar results were obtained by test drug (Group II) and standard drug (Group III). These changes in biochemicals of Group II and III were insignificant as compared with diabetic control (Group I) [Table 4].

DISCUSSION

Diabetes mellitus greatly influences utilization of carbohydrates, which in turn leads to imbalance in metabolism of lipids and proteins. Therefore, in management of diabetes, decreasing abnormally elevated blood glucose concentration to normal limits is of utmost importance. Among many forms of Diabetes mellitus,

Table 1: Acute study on normal rats

Gr. no.	Groups	Mean blood glucose levels in mg/dl ± standard error			
		0 Hr	2 Hrs	3 Hrs	4 Hrs
I	Normal control	91.83 ± 4.9	79.33 ± 5.8 (13.61)	74.17 ± 7.4 (19.24)	72.83 ± 8.9 (20.69)
II	*C. javanica* 0.5g/kg b.w.	80.50 ± 9.5	73.50 ± 3.0 (8.70)	57.50 ± 6.0 (28.57)	56.67 ± 9.6 (29.61)
t values		1.06	0.88	1.75	1.24

n=6 in each group, df = 10, Table $t_{[0.05]}$ = 2.228; Values in parentheses indicate % reduction in glucose level as compared with 0 Hr

Table 2: Acute study on diabetic rats

Gr. no.	Groups	Mean blood glucose levels in mg/dl ± standard error			
		0 Hr	2 Hrs	3 Hrs	4 Hrs
III	Diabetic control	481.33 ± 27	481.67 ± 11.6 (-0.07)	486.17 ± 28.7 (-1.00)	484.17 ± 26.1 (-0.59)
IV	*C. javanica* 0.5g/kg b.w.	449.67 ± 14.0	433.83 ± 14.3 (3.53)	430.17 ± 13.3 (4.34)	422.17 ± 12.8 (6.12)
V	Standard 0.01g/kg b.w.	482.33 ± 5.5	460.17 ± 14.9 (4.59)	413.5 ± 13.9 (14.27)	382.33 ± 11.8 (20.73)* P<0.05
F values		0.91	1.40	3.64	8.01
$CD_{[0.05]}$					66.74
$CD_{[0.01]}$					87.83

n=6 in each group, df_1=2 and df_2=15, Table $F_{[0.05]}$ = 3.68; Values in parentheses indicate % reduction in glucose level as compared with 0 Hr; *indicates significant results P<0.05 in comparison with Diabetic Control Group

Table 3: Sub-acute study on diabetic rats

Gr. no.	Groups	Mean blood glucose levels in mg/dl ± standard error					
		0 Day	2 Days	4 Days	6 Days	8 Days	10 Days
I	Diabetic control	406.17 ± 11.9	409.17 ± 7.0 (–0.74)	408.67 ± 10.0 (–0.62)	407.67 ± 7.6 (–0.40)	407.83 ± 9.6 (–0.41)	408.00 ± 9.1 (–0.45)
II	*C. javanica* 0.5g/kg b.w.	454.50 ± 25.0	440.50 ± 24.0 (3.08)	416.83 ± 31.0 (8.29)	363.83 ± 23.0 (19.95)	336.50 ± 20.4 (25.96)* $P<0.05$	283.50 ± 13.5 (37.62)** $P<0.01$
III	Standard 0.01g/kg b.w.	382.33 ± 11.0	271.00 ± 7.6 (29.12)** $P<0.01$	219.33 ± 9.7 (42.63)** $P<0.01$	182.67 ± 18.0 (52.22)** $P<0.01$	157.67 ± 12.0 (58.76)** $P<0.01$	139.50 ± 5.2 (63.51)** $P<0.01$
F values		4.41	34.68	32.27	45.66	76.00	185.77
$CD_{[0.05]}$		64.43	56.36	72.40	64.97	54.14	36.28
$CD_{[0.01]}$		84.79	74.18	95.28	85.50	71.60	47.75
IV	Normal control	97.83 ± 2.2	102.67 ± 5.3 (–4.95)	107.67 ± 1.8 (–10.06)	105.33 ± 3.7 (–7.67)	105.00 ± 2.76 (–7.33)	103.83 ± 2.07 (–6.13)

$n=6$ in each group, $df_1=2$ and $df_2=15$, Table $F_{[0.05]}=3.68$; Values in parentheses indicate % reduction in glucose level as compared with 0 Day; * and ** indicate significant results $P<0.05$ and $P<0.01$ in comparison with Diabetic Control Group respectively

Table 4: Sub-acute study of biochemical parameters of diabetic rats

Gr. no.	Groups	Biochemical parameters (Mean values±standard error)					
		Total cholesterol (mg/dl)		Triglycerides (mg/dl)		Total proteins (g/dl)	
		0 Day	10 Days	0 Day	10 Days	0 Day	10 Days
I	Diabetic control	70.33 ± 4.4	76.00 ± 4.6	98.17 ± 5.1	99.00 ± 3.5	5.72 ± 0.2	5.13 ± 0.1
II	*C. javanica* 0.5g/kg b.w.	58.83 ± 4.3	69.67 ± 3.2	93.50 ± 5.8	91.17 ± 3.6	5.57 ± 0.2	4.98 ± 0.2
III	Standard 0.01g/kg b.w.	59.67 ± 4.5	60.17 ± 5.1	86.50 ± 4.7	85.83 ± 6.6	5.57 ± 0.2	5.53 ± 0.2
	F values	2.13	3.34	1.27	1.91	0.25	3.65
IV	Normal Control	31.52 ± 3.0	37.69 ± 4.2	74.65 ± 10.0	79.15 ± 4.0	8.58 ± 0.3	8.63 ± 0.3

$n=6$ in each group, $df_1 2$ = and $df_2=15$ Table $F_{[0.05]}=3.68$

Type II (Non -Insulin -Dependent Diabetes mellitus) occurs predominantly and affects major population, i.e., 90% of diabetic patients.[24] STZ selectively causes damage of insulin-producing pancreatic β-cells of rats. This in turn induces diabetic condition that mimics the Type II diabetes of human beings. It produces abnormal levels of blood glucose and serum metabolites like cholesterol, triglycerides, and proteins. Hence, these major parameters were taken into consideration while investigating hypoglycemic effect of *C. javanica*.

The hypoglycemic effect of the drug was observed only in sub-acute treatment. Probably, the effective concentration of active antidiabetic principles was not achieved by single dose. Hence, in comparison with single-dose treatment, multiple doses of drug showed remarkable activity. The hypoglycemic effect of the drug might be the result of the synergistic action of antidiabetic active principles of leaves. The drug might be responsible for stimulation of insulin release and restoration of normal blood glucose level of diabetic animals. Furthermore, the obtained blood glucose reduction may be possibly due to increased peripheral glucose utilization. Often, in Diabetes mellitus, deranged levels of lipids and proteins are of major concern as they cause severe metabolic complications. Although the test drug was effective against hyperglycemia, it proved to be non-functional in correcting the abnormal levels of serum total cholesterol, triglycerides, and total proteins.

CONCLUSION

Currently, insulin and synthetic oral hypoglycemic agents like sulfonylureas and biguanides are the major players in management of Diabetes mellitus. Despite the availability of synthetic drugs, there is an ever-increasing demand of antidiabetic herbal options. Through present work, one novel leafy drug of *C. javanica* has been proved to be hypoglycemic agent. In comparison with standard drug, it showed less activity, but still it was quite significant. Even though this drug has not affected deranged serum biochemicals, it can be used in polyherbal formulations. In combination with other hypolipidemic and protein rich herbal options, drug may correct metabolic anomaly also. In conclusion, *C. javanica* leaf is safe and effective oral hypoglycemic agent. Further studies are necessary to elucidate details of active phytochemicals and their mechanism of hypoglycemic action. Work is in progress to isolate active principles.

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