ORIGINAL ARTICLE

Evaluation of hypoglycaemic activity of *Cassia nodosa* leaves in normal and streptozotocin-induced diabetic rats

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In present work, one novel ornamental plant *Cassia nodosa* Buch.-Ham. ex Roxb. has been investigated for hypoglycaemic action. It aimed to check the hypoglycaemic effect of *C. nodosa* leaves on normal and streptozotocin-induced diabetic rats by acute and sub-acute studies. Prior to the hypoglycaemic 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 streptozotocin. 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 hypoglycaemic drug-glibenclamide (0.01 g/kg/day). It was done by 'Student's '*t*' test' and one-way ANOVA test. In preliminary phytochemistry antidiabetic compounds were detected. Unlike acute, sub-acute treatment of test drug showed highly significant reduction (40.29%) in blood glucose level of diabetic rats in 10 days. This effect was considerably good in comparison with standard drug (63.51%). The test drug and standard drug exhibited an insignificant change in the abnormal levels of serum metabolites of diabetic rats. Preclinically *C. nodosa* was proved to be an effective hypoglycaemic agent.

Key words: Acute toxicity, *Cassia nodosa* leaves, hypoglycaemic, streptozotocin

INTRODUCTION

In recent years, Diabetes mellitus (DM) has become a serious global health problem affecting about 10% population of the world.[1] DM is mainly characterized by abnormally elevated blood glucose level. It also shows altered metabolism of carbohydrates, lipids, and proteins with an increased risk of vascular and renal diseases.[2,3] Presently insulin and synthetic oral hypoglycaemic agents are the major players in management of it.[1] Frequently herbal medication is also preferred as one of the alternative methods. 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 preclinical and clinical studies of various herbal crude drugs are essential in development of complete antidiabetic drug. Keeping this view in mind, in the present investigation one of the unexplored plant *Cassia nodosa* had been studied for hypoglycaemic action.

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Cassia nodosa (Pink Cassia) is a popular garden plant from family Leguminosae. It is a medium-sized tree that cultivated throughout India for beautiful blossoms of pink flowers. Therapeutic uses of the plant are not discussed in detail in previous literature. However it is reported that, leaves are edible and medicinally used for poulticing boils.[4,5] They contain alkaloids, glycosides, and flavonoids.[4,6] These compounds are considered to be antidiabetic.[7,8] Considering the potential leaf constituents of *C. nodosa*, bioactivity of the powdered leaf drug was checked against streptozotocin-induced diabetes in rats.

MATERIALS AND METHODS

Plant Material

The samples of leaves of *Cassia nodosa* were procured from various regions of Mumbai and Pune. The mature leaves were obtained during their flowering season of May to July. The botanical identity was confirmed using the standard herbaria at Blatter Herbarium of St. Xavier's College, Mumbai (Accession No. Blat. 15780). Leaf samples were subjected to artificial drying at 40°C and ground to form powder. The powdered

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drug samples were moderately coarse as they were seivable through mesh no. 710 with 0.710 mm size of aperture.[9,10] It was stored in closed, airtight containers with silica bags.

Preliminary Phytochemical Analysis of Drug

A total of 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.[11,12]

Animals

Laboratory bred male Wistar albino adult rats weighing 200–250 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 (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

The acute toxicity study was carried out as per the procedure given in OECD Guideline No. 420.[13] The male Wistar albino rats (200-250 g) were used in the study. After the sighting study, the drug of *C. nodosa* 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 behaviour. No change in behaviour and death were 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 Streptozotocin (STZ) in a 0.1 M Citrate buffer (pH 4.5) was given to them. The fasting blood glucose levels were checked after 3 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.[14,15]

Acute Study on Normal Rats

To determine the hypoglycaemic activity of the drug, normoglycaemic 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. nodosa* at oral dose of 0.5 g/kg body weight in vehicle. The samples of blood were obtained zero, second, third, and fourth hour of the treatment. The blood glucose levels were determined using a glucometer.[16,17]

Acute Study on Streptozotocin induced Diabetic Rats

For testing drug activity on diabetic rats, 18 hour 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. nodosa* at the dose of 0.5 g/kg body weight in vehicle. Dosing of 0.01 g/kg body weight of the standard oral hypoglycaemic 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 hours of the treatment. The blood glucose levels were determined using a glucometer.[16,17]

Sub-acute Study on Diabetic Rats

In the sub-acute method, three groups of diabetic rats namely groups I, II, and III were made in a similar way to the acute study. 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 10 days. During the study, fasting blood glucose levels were checked on zero, second, fourth, sixth, eighth, and tenth days of experiment.

The biochemical parameters such as total cholesterol, triglycerides, and total proteins were estimated from serum samples on the 0^{th} and 10^{th} days. The samples of serum were obtained from blood withdrawn from retroorbital plexus. Commercial kits of Agappe Diagnostics were used for biochemical estimations.[18,19]

Statistical Analysis

The values of all parameters were expressed as Mean±Standard Error in tables. The data were statistically analysed by 'Student's '*t*' test' and one-way ANOVA test. *P* values <0.05 and <0.01 were considered to be significant.[20]

RESULTS

The preliminary phytochemical analysis of *C. nodosa* leaves exhibited the presence of reducing sugars, proteins, alkaloids, tannins, glycosides, sterols, flavonoids, and saponins. As some of these compounds (alkaloids,

glycosides, sterols, flavonoids and saponins) are known to possess antidiabetic properties, the hypoglycaemic study of the said drug has been carried out for the first time.

In the acute oral toxicity study, at a dose of 2 g/kg body weight of *Cassia nodosa* mortality was not observed. The animal behaviour was also found to be unchanged. Therefore 0.5 g/kg body weight dose of drug was considered safe and used for further investigation.

In acute treatment the effect of single dose (0.5 g/kg body weight) of test drug was checked till the fourth hour of drug administration. Statistical analysis of data showed that test drug produced insignificant hypoglycaemic effect in both normal (group II) and diabetic rats (group IV) compared to normal control (group I) and diabetic control (group III) respectively. But at the same time interval standard drug (group V) exhibited a quite significant (P<0.05) result with 20.73% reduction in comparison with diabetic control [Tables 1 and 2].

Unlike acute, sub-acute treatment of test drug demonstrated excellent hypoglycaemic activity. test drug at the dose of 0.5 g/kg body weight/day started significant action (P<0.05) from the sixth day of drug administration (group II). In short 10 days' treatment drug induced a consistent decrease in glucose concentration and total reduction of 40.29% 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 glibenclamide (group III) caused significant (P<0.01) fall in glucose level from the second day with total reduction of 63.21% in 10 days' treatment. Although test drug showed delayed response but it showed quick reduction. The results of test and standard drugs were found to be comparable [Table 3].

Table 1: Acute study on normal rats

Gr. no.	Groups	Mean blood glucose levels in mg/dl±standard error			
		0 hour	2 hours	3 hours	4 hours
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. nodosa* 0.5 g/kg b.w.	81.33±5.1	73.50±3.0 (9.63)	63.50±3.0 (21.92)	57.50±1.9 (29.30)
t values		1.48	0.88	1.34	1.68
P values		0.169	0.399	0.209	0.123

n=6 in each group, df=10, Table $t_{[0.05]}$=2.228. Values in parentheses indicate % reduction in glucose level compared to 0 h

Table 2: Acute study on diabetic rats

Gr. no.	Groups	Mean blood glucose levels in mg/dl±standard error			
		0 hour	2 hours	3 hours	4 hours
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. nodosa* 0.5 g/kg b.w.	461.50±22.0	453.17±9.5 (1.80)	430.33±25.7 (6.75)	419.83±26.7 (9.03)
V	Standard 0.01 g/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)*
F values		0.29	0.42	2.59	5.17
$CD_{[0.05]}$					83.33
$CD_{[0.01]}$					109.66
P values		0.752	0.664	0.180	0.019

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 compared to 0 hour. *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. nodosa* 0.5 g/kg b.w.	419.83±26.0	394.17±29.0 (6.11)	364.67±24.0 (13.14)	320.50±26.0 (23.66)*	304.50±24.0 (27.47)**	250.67±14.9 (40.29)**
III	Standard 0.01 g/kg b.w.	382.33±11.0	271.00±7.6 (29.12)**	219.33±9.7 (42.63)**	182.67±18.0 (52.22)**	157.67±12.0 (58.76)**	139.50±5.2 (63.51)**
F values		1.08	17.25	36.68	34.27	57.34	165.15
$CD_{[0.05]}$			67.18	60.21	71.33	61.10	38.64
$CD_{[0.01]}$			88.14	79.24	93.87	80.41	50.85
P values		0.364	0.000129	0.00000167	0.00000255	0.00000009	0.0
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 compared to 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 (gm/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. nodosa* 0.5 g/kg b.w.	69.00±3.9	69.67±3.2	88.67±6.6	84.33±3.7	5.57±0.1	4.98±0.2
III	Standard 10 mg/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		1.87	3.34	1.27	2.79	0.29	3.65
P values		0.188	0.063	0.309	0.093	0.752	0.051
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

Sub-acute treatment also involved testing of serum biochemicals. In diabetic rats the levels of total cholesterol and triglycerides were abnormally elevated and total proteins level was noticeably reduced as compared to normal control (group IV). Biochemical assays were done on the 0^{th} and 10^{th} days. During the course of treatment in diabetic control (group I), there was a slight increase in levels of total cholesterol and triglycerides while total protein level was lowered. Almost similar results were obtained for test drug (group II) and standard drug (group III). Statistically, these changes in biochemicals of groups II and III were insignificant compared to diabetic control (group I) [Table 4].

DISCUSSION

DM is mainly characterized by hyperglycaemia and often associated with hyperlipidaemia and hypoproteinaemia. Among many forms of DM, type II (non-insulin-dependent diabetes mellitus) occurs predominantly and affects major population, i.e., 90% of diabetic patients.[21] Streptozotocin 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. It also produces raised levels of cholesterol and triglycerides as well as reduced level of total proteins. Therefore in order to know the effects of test drug, levels of blood glucose, and serum biochemicals were checked at specific time interval after drug administration.

Repeated administration of test drug in sub-acute treatment proved to be very useful and produced remarkable hypoglycaemic activity. Probably the effective concentration of active antidiabetic principles was achieved in multiple doses only. Drug was in crude form hence synergistic action of antidiabetic principles of leaves might have produced the hypoglycaemic effect. As β-cells were destroyed it was clear that the hypoglycaemic effect may be due to potentiating insulin from few existing β-cells or extrapancreatic mechanism. Perhaps increased peripheral glucose utilization also involved in blood glucose reduction.

In DM, frequently abnormal levels of lipids and proteins cause serious metabolic complications. This is due to deficiency of insulin which controls various metabolic pathways. The present study provided substantial evidence for hypoglycaemic action of all the drugs but they proved to be non-functional in correcting deranged levels of triglycerides, total cholesterol and total proteins. The most likely reason can be ineffectiveness of drugs towards enzymes that cause lipid mobilization and protein catabolism.

CONCLUSION

Herbal hypoglycaemic agents can provide better option to avoid harmful side effects caused by prolong intake of synthetic ones. From present preclinical studies, *Cassia nodosa* proved to be hypoglycaemic in action. But one can speculate that in clinical trials, the drug may act as safe and effective hypoglycaemic agent for mankind also. The remarkable hypoglycaemic potential of *C. nodosa* was quite competent with standard drug. Although the test drug could not correct deranged levels of serum metabolites, it can be used in polyherbal formulations. Further studies are necessary to elucidate details of active phytochemicals and their mechanism of hypoglycaemic action. Isolation and study of active principles are under process.

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