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RESEARCH ARTICLE

Evaluation of Antidiabetic Activity of Cassia Glauca Lam. Leaf in Streptozotocin Induced Diabetic Rats

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ABSTRACT

The objective of the study was to investigate the antidiabetic action of aqueous extract of *Cassia glauca* leaf in different models of rats. The antidiabetic activity of aqueous extract of *Cassia glauca* leaf was evaluated by using normal and streptozotocin-induced diabetic rats. After the oral administration of aqueous extracts at doses of 500 mg/kg body weight, blood glucose levels and body weights were monitored at specific intervals. Glibenclamide was used as a reference drug at a dose of 0.25 mg/kg mg/kg. The various parameters studied included serum lipid levels, liver glycogen content, serum insulin level, urea creatinine, total hemoglobin and glycosylated hemoglobin in diabetic and normal rats. On oral administration of aqueous extract of *Cassia glauca* leaf at a dose of 500 mg/kg, normoglycemic rats did not show any significant effect on blood glucose levels, whereas aqueous extract showed statistically significant effect ($p < 0.001$) by reducing the effect of external glucose load. In chronic model of diabetic, aqueous extract of *Cassia glauca* leaf at a dose of 500 mg/kg and glibenclamide (0.25 mg/kg) were administered for 21 days. At the end of treatment, there was significant increase in the body weight, liver glycogen, serum insulin level and the HDL cholesterol levels. There was a significant decrease in fasting blood glucose, glycated hemoglobin, total cholesterol and serum triglycerides. Our results suggest that *Cassia glauca* leaf have potent antidiabetic property, justifying the use of drug for the treatment of diabetes mellitus.

Keywords: *Antidiabetic, Cassia glauca, Glibenclamide, Lipid profile, Serum level*

In the recent years traditional or complementary medicine has seen an upsurge and according to two studies, almost 48.5% Australian respondents, and 34% of American respondents had used at least one form of unconventional therapy including herbal medicine [1,2]. Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The increasing number of ageing population, consumption of calories rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetic patients worldwide. The current treatment although provides good glycemic control but does a little in preventing complications. Besides, these drugs are associated with side effects [3]. Moreover, providing modern medical healthcare across the world (especially in developing countries such as India), it is still a far-reaching goal due to economic constraints. Thus, it is necessary that we continue to look for new and if possible more efficacious drugs and the vast reserves of phytotherapy may be ideal target [3].

Cassia glauca Lam. is a glabrous tree belonging to family Caesalpiniacea, found throughout India, tropical Asia and Australia. The leaves are long linear, acute, curved in shape. The flower is yellow in color and shorter than the leaves [4]. Phytochemical study of stem of *Cassia glauca* has been indicated the presence of chrysophenol, physcion, stearic acid, β-sitosterol and β-D glucoside [5]. In folk medicine, bark and leaves of *Cassia glauca* are used for the treatment of diabetes and gonorrhea. The leaves are used for blennorrhagia [4]. In Ayurvedic systems of medicine, herbal extracts but not purified compounds have been used from centuries because of many constituents are considered to be beneficial. Here, we report detail studies on antidiabetic activity of the aqueous extract from leaves of *Cassia glauca*, with a view to provide scientific evidence of modern lines.

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MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Cassia glauca* Lam. were collected during November 2007 from the Western Ghats, Karnataka, India. The leaves were identified by Dr. Harsha Hegde, Research Officer, RMRC, ICMR, Belgaum, Karnataka, India. A voucher specimen (SA-02) has been kept in herbarium of Department of Pharmacognosy, KLES College of Pharmacy, Belgaum, Karnataka, India.

Preparation of Aqueous Extract of Leaves

Cassia glauca leaves were air dried in the shade and cut into small pieces. Hundred grams of leaves were extracted with 1000 ml of water by the method of hot extraction at 40-60ºC for 6 h. Water was evaporated to get dry extract. The extract was dissolved in water and used in the study [6]. A weighed portion of extract was suspended in 0.5% aqueous carboxymethylcellulose (CMC) solution in distilled water prior to oral administration to animals.

Animals

Healthy *Wistar* rats between 2 and 3 months of age and weighing 180–200 g were used for the study. House individually in polypropylene cages, maintaining under standard conditions (12 h light and 12 h dark cycle), and the animals was fed with standard rat pellet diet and water ad libitum. The study was approved by Institutional Animal Ethical Committee of Jawaharlal Nehru Medical College, Belgaum, India (CPCEA No. 221, Resolution No. 1/16/2007).

Acute Toxicity Studies

Healthy *Wistar* rats of either sex were used, starved overnight were orally fed with the aqueous extracts in increasing dose levels of 500, 1000, 3000 and 5000 mg/kg body weight. The animals were observed continuously for 2 h under the following profiles:

(i) *Behavioral profile*: Alertness, restlessness, irritability and fearfulness

(ii) *Neurological profile*: Spontaneous activity, reactivity, touch response, pain response and gait

(iii) *Autonomic profile*: Defecation and urination.

After a period of 24 and 72 h animals were observed for any lethality or death [7].

Effect of Aqueous Extract in Normoglycemic Rats (NG)

Each group consisted of six rats. Fasting blood sugar level of each animal was determined after overnight fasting for 16 h. The animals in control group received saline. The test group of animals was treated with the aqueous extract of *Cassia glauca* leaves (500 mg/kg p.o.). Blood samples were collected at 30, 60 and 120 min after the oral administration of aqueous extract [7].

Oral Glucose Tolerance Test in Normal Rats (OGTT)

The oral glucose tolerance test was performed in overnight-fasted (18 h) normal rats. Rats divided into two groups (n=6) were administered drinking water and 500 mg/kg aqueous extract, respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood will be withdrawn at 30, 60 and 120 min of glucose administration and glucose levels were estimated using a glucose oxidase–peroxidase reactive strips and a glucometer (Sugarcheck, Wockhardt Ltd., Mumbai, India) [7].

Evaluation of Antidiabetic Activity [7-11]

Induction of Diabetes

Diabetes was induced in rats by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 70 mg/kg b.w., dissolved in 0.1 M cold citrate buffer (pH= 4.5). Seven days after the injection, the blood glucose concentration level above 200 mg/dl was considered to be diabetic and used in the experiments. To prevent the hypoglycemia which occurred during the first 24 h following the STZ administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were fasted for 16 h prior to STZ injection. Only rats found with permanent diabetic were used for the antidiabetic study.

Determination of Hypoglycemic Activity on Acute Administration

The diabetic rats exhibiting blood glucose levels in the range of 200 and 300 mg/100 ml were selected for the studies. These diabetic rats were sub-divided into 2 groups as follows:

Group 1: Diabetic control. Received only vehicle (saline)

Group 2: Diabetic rats given (500 mg/kg b.w. p.o.) aqueous extract of *Cassia glauca* leaf

The blood glucose concentrations of the animals were measured at the beginning of the study and the oral glucose tolerance test was performed in overnight-fasted (18 h) diabetic rats. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood will be withdrawn at 30, 60 and 120 min of glucose administration and glucose levels were estimated using a glucose oxidase–peroxidase reactive strips and a glucometer (Sugarcheck, Wockhardt Ltd., Mumbai, India).

Chronic Treatment Model

Four groups of six rats each were used in the experiment. Group 1 served as normal healthy control group and group 2 as diabetic untreated control. Group 3 was treated with 500 mg/kg b.w. aqueous extract of *Cassia glauca* leaves for 21 day and the water was given to the control group animals. Group 4 served as standard and was treated with glibenclamide (0.25 mg/kg p.o.). Body weight measurements were done on day 1, 7 and 21 day of the study. On day 21, blood was collected under mild ether anesthesia from overnight

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Table 1. Hypoglycemic effect of aqueous extract in different models of rats

Test model	Group	Dose (mg/kg)	Mean blood glucose concentration (mg/dl) ± S.E.M			
			0 h	30 min	60 min	120 min
NG	Control	----	72.0 ± 3.2	97.7 ± 2.0	101.5 ± 2.5	88.5 ± 2.5
	Aqueous extract	500	77.9 ± 5.9	99.6 ± 7.1	98.5 ± 2.7	76.5 ± 1.6
OGTT	Control	-----	81.5 ± 4.8	97.7 ± 2.0	145.0 ± 2.9	107.0 ± 2.5
	Aqueous extract	500	84.0 ± 4.9	96.0 ± 4.0	137.0 ± 3.9	98.0 ± 2.5
Diabetic	Diabetic control	----	380.5 ± 15.3	354.8 ± 19.2	365.7 ± 17.5	399.2 ± 20.9
	Aqueous extract	500	430.2 ± 31.9	481.2 ± 20.7	399.8 ± 17.6**	241.5 ± 2.0***

Values are given as mean ± S.E M from six rats in each group
***p < 0.01* most significant, ****p < 0.001* highly significant when compared with diabetic control animals

Table 2. Effect of aqueous extract on body weight of rats

Group	Treatment	Body weight changes		
		1st day	7th day	21st day
I	Normal control	185.2 ± 3.35	196.3 ± 2.28	230.3 ± 2.15
II	Diabetic control	221.0 ± 7.70	218.3 ± 8.16	159.5 ± 5.54
III	Diabetic + Glibenclamide (0.25 mg/kg)	156.3 ± 9.19	172.0 ± 8.39	211.2 ± 6.63***
IV	Diabetic + Aqueous extract (500 mg/kg)	215.3 ± 3.90	188.3 ±2.27	193.2 ± 2.12 ***

Values are given as mean ± S.E for six rats in each group
**p < 0.001* highly significant from diabetic control animals

fasted rats. Serum glucose levels, total cholesterol, HDL cholesterol, triglyceride levels in serum, VLDL and LDL and serum insulin were estimated.

Statistical Evaluation

Values are presented as mean ± S.E.M. Statistical difference between the treatment and the controls were tested by using one-way analysis of variance (ANOVA) followed by Student's *t* test (500 mg/kg drug treated group was compared with control). A p value <0.05 was considered significant.

RESULTS

Acute Toxicity Study

Experiments were carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal. Therefore, the cut off dose for effective dose (ED_{50}) was taken as 500 mg/kg b. w. which is the $1/10^{th}$ of LD_{50}.

Hypoglycemic Effect of Aqueous Leaf Extract

As a preliminary activity assessment, the aqueous extract was prepared and administered to normal, glucose-hyperglycemic and STZ-induced diabetic rats at a dose of 500 mg/kg b.w. to determine the acute effect on the blood concentrations. Changes in the blood glucose level of each group of animal were followed during a 2 h period. Consequently, the extract showed significant hypoglycemic activity in OGTT and STZ-diabetic rats, while no remarkable effect was observed on normoglycemic rats (Table 1). The data obtained revealed that the aqueous extract possessed a remarkable hypoglycemic effect at 500 mg/kg dose in diabetic rats.

Changes of Body Weight

Polyuria is known as one of the common complications in diabetics and induces a loss in body weight. In order to monitor the effect of aqueous extract of *Cassia gluca* leaf on body weight in diabetic rats during the acute administration, the body weight of each animal was recorded on 1st, 7th and 21st days. There was significant increase in body weight observed on day 21 with aqueous extract of *Cassia gluca* as compared to control. Body weight of the animals in standard drug glibenclamide-treated group also increased significantly on day 21 (Table 2).

Changes of Serum Glucose Level in Chronic Model

The effect of aqueous extract of *Cassia glauca* leaf on fasting plasma glucose levels are presented in Table 3. The difference between the experimental (*Cassia glauca* leaf extract) rats in lowering the fasting plasma glucose levels was statistically significant ($p < 0.001$) as compared in diabetic rats on day 21 from day 1. Standard drug also lowered the serum glucose level significantly.

Changes of Serum Insulin, Liver Glycogen and Glycated Hemoglobin

Since the aqueous extract of *Cassia glauca* leaf showed significant improvement in fasting blood glucose and OGTT of diabetic animals, it was intended to assess the effect of long-term treatment of the extract on serum insulin, liver glycogen and glycated hemoglobin in STZ-induced chronic diabetic rat model. Rats were treated with 500 mg/kg b.w. of aqueous extract once a day in the morning for 21 day. At the end

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Table 3. Effect of aqueous extract on serum glucose level in chronic model

Group	Treatment	Fasting plasma glucose concentration (mg/dL)		
		1st day	7th day	21st day
I	Normal control	77.5 ± 4.3	86.172 ± 3.8	93.52 ± 3.9
II	Diabetic control	259.3 ± 7.4	355.5 ± 18.1	499.0 ±10.0
III	Diabetic + Glibenclamide (0.25 mg/kg)	294.5 ± 11.0	378.0 ± 32.9	212.21 ± 6.8***
IV	Diabetic + Aqueous extract (500 mg/kg)	249.0 ± 7.8	305.3 ± 13.5	284.5 ± 10.5***

Values are given as mean ± S.E from six rats in each group
****p < 0.001* significant from diabetic control animals

Table 4. Effect of aqueous extract on serum insulin, liver glycogen and glycated hemoglobin levels in diabetic rats

Group	Treatment	Serum insulin (ng/ml)	Glycated hemoglobin (%)	Liver glycogen (mg/g)
I	Normal control	0.25 ± 0.22	3.23 ± 0.09	12.76 ± 2.31
II	Diabetic control	0.18 ± 0.01	6.68 ± 0.16	5.06 ± 0.11
III	Diabetic + Glibenclamide (0.25 mg/kg)	0.32 ± 0.02**	4.80 ± 0.27***	13.98 ± 0.12*
IV	Diabetic +Aqueous extract (500 mg/kg)	0.29 ± 0.00**	3.35 ± 0.24***	12.64 ± 0.71*

Values are given as mean ± S.E.M from six rats in each group
**p < 0.05* significant, ***p< 0.01* most significant, ****p< 0.001* highly significant from diabetic control animals

Table 5. Effect of aqueous extract on serum lipid profile in diabetic rats

Group	Treatment	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)
I	Normal control	78.50 ± 3.33	54.83 ± 0.60	16.17 ± 0.47
II	Diabetic control	117.80 ± 5.37	143.70 ± 6.63	15.17 ± 0.30
III	Diabetic + Glibenclamide (0.25 mg/kg)	83.50 ± 1.23***	88.50 ± 3.49 ***	16.83 ± 0.60*
IV	Diabetic +Aqueous extract (500 mg/kg)	84.67 ± 3.88***	89.17 ± 2.02 ***	16.33 ± 0.49*

Values are given as mean ± S.E from six rats in each group
**p < 0.05* significant, ****p < 0.001* highly significant from diabetic control animals

of the treatment, the animals when compared with diabetic control, showed significant (*p < 0.01)* difference in serum insulin level and glycated hemoglobin level and significant (*p < 0.05)* effect on liver glycogen level (Table 4).

Lipid Profile

The various parameters of blood lipid profile of severely diabetic control rats were compared with treatment group. The enhanced levels of Total cholesterol, LDL, VLDL and triglyceride were brought down significantly (*p < 0.001)* after the treatment period. There was also statistically significant (*p < 0.05)* increase of HDL cholesterol in the treated diabetic rats. In untreated diabetic rats there was a fall in HDL (Table 5).

DISCUSSION AND CONCLUSIONS

Ayurvedic system of medicine relies on the administration of crude extract or concentrated extract of the plant material for the treatment of diabetes mellitus. Preliminary studies demand further research so that their novel possibilities as a source of oral hypoglycemic agents could be investigated [12,13]. Literature survey indicates that there is no scientific evidence to support the antidiabetic effect of *Cassia glauca* Lam. Therefore the present study is undertaken to investigate the action of aqueous extract of *Cassia glauca* leaves in different models of rats to ascertain the scientific basis for the use of these plants in the treatment of diabetes.

The present investigation reports the hypoglycemic and antidiabetic effect of aqueous extract *Cassia glauca* leaves. The observations reported here offer scientific explanation for the potential use of this plant for the treatment of diabetes. Overall results shows that aqueous extract of leaves possess marked hypoglycemic activity by improvement of glucose tolerance test and by lowering the blood glucose levels in STZ-induced diabetic rats. The hypoglycemic effect of aqueous extract on normoglycemic rats was not significant.

In chronic diabetic model, physically it was observed that body weight of the rats in aqueous extract-treated group increased significantly (*p < 0.001)* after the completion of 21 days treatment. This effect was quite similar with that of standard drug glibenclamide. Hence, it can be said that *Cassia glauca* leaves does not have any effect on degradation of depot fat and it can maintain the body weight in type 2

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diabetic state. Serum glucose level was 305.3±13.5 mg/dl on day 7 and it decreased significantly ($p < 0.05$) to 284.5±10.5 mg/dl with aqueous extract of *Cassia glauca* leaves. This phenomenon clearly indicates that extract of *Cassia glauca* leaves can potentially control the hyperglycemic state of diabetes.

Administration of aqueous extract of *Cassia glauca* showed significant ($p < 0.01$) increase in the levels of serum insulin. The possible mechanism by which aqueous extract brings about its hypoglycemic action in diabetic rats may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. A marked increase in total cholesterol and decrease in HDL cholesterol have been observed in untreated diabetic rats.

Clinically, it has been observed that there is altered fat metabolism in type1 and type 2 diabetes leading to altered serum cholesterol and triglyceride level [14]. Hypercholesterolemia and hypertryglyceredemia have been reported to occur in streptozotocin-induced diabetic rats [15]. Under normal circumstances, insulin activates enzyme lipoprotein lipase and hydrolyses triglycerides. Insulin deficiency results in failure to activate the enzymes thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the aqueous extract-treated diabetic rats may be directly attributed to improvements in insulin levels upon *Cassia glauca* extract therapy.

Induction of diabetes with STZ is associated with the characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins [16,17]. Diabetic rats treated with the aqueous extract of *Cassia glauca* leaves showed an increase in body weight as compared to control, which may be due to its protective effect in controlling muscle wasting i.e. reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control.

The significant increase in the glycogen level of the aqueous extract-treated diabetic animals may be because of the reactivation of glycogen synthase system. This focuses the one possible way of antidiabetic action of this extract by improvement of glycogenesis process [18]. Glycated hemoglobin levels were found to be increased in the untreated diabetic control group. Treatment with aqueous extract of *Cassia glauca* leaves showed a significant decrease in the glycated hemoglobin levels, which could be due to an improvement in insulin secretion, which confirms the antidiabetogenic action of the extract. The extract did not produce any significant effects on normal animals. Further studies of *Cassia glauca* and its isolated compounds are necessary to elucidate the exact mechanism of action so as to develop it as a potent antidiabetic drug.

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