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Hypoglycemic and antidiabetic screening of *Cassia fistula* Linn. Bark

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ABSTRACT

Animals were treated with alcoholic and aqueous extract of *Cassia fistula* at a dose level of 400mg/kg bodyweight by standard methods. Blood glucose, glycosylated hemoglobin, triglyceride, LDL, VLDL, HDL, blood urea and cholesterol were measured at the beginning and at the end of the experiment. The extracts have shown potential activity in decreasing the serum glucose level and other complications associated with experimental diabetes. The extracts also showed significant hypoglycemic effect in fasted normal rats (P<0.01). The present study reveals that the bark of *Cassia fistula* is very promising for developing standardized phytomedicine for diabetes mellitus. Thus the present study supports the traditional claim of the plant for the ailment of various diabetes associated complications.

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KEYWORDS

Hypoglycemic;
Antidiabetic;
Cassia fistula.

INTRODUCTION

Diabetes mellitus, a chronic metabolic disorder, has now become an epidemic, with a worldwide incidence of 5% in the general population. The number of people suffering from diabetes has soared to 246 million and the disease now kills more people than AIDS[1]. Decreased physical activity, increasing obesity, stress and changes in food consumption have been implicated in this increasing prevalence in the past two decades[2]. Though different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes, there is an increased demand by patients to use natural products with antidiabetic activity[3]. Recently, more works are being published on the antidiabetic activities of various plants in response to WHO recommendation of diabetes mellitus.

The plant *Cassia fistula* (Leguminosae) gained its name as "Indian Laburnum" is a perennial tree found in India[4]. It is known as Konnei in Tamil[5]. The plant possess innumerable therapeutic activities such as emetic, cathartic astringent, tonic, febrifuge, purgatives, laxatives, antidiabetic, antidote for snake bite, in the treatment of gout, rheumatism and antitumour[6]. The presence of active constituents viz. barbaloin, fistucacidin, an optically inactive leucoanthocyanidin, 3,4,7,8,4'-pentahydroxyflavan and anthroquinone glycoside-rhein have been reported from the bark[7]. Earlier studies showed that ethanolic extract of both root and fruit reported for its antidiabetic activity. Since no scientific proof about antidiabetic activity of *Cassia fistula* bark, an attempt has been made to explore such activity for *Cassia fistula* bark. In the present work, alcoholic and aqueous extracts of *Cassia fistula* bark

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were evaluated for hypoglycemic and antidiabetic activity (alloxan induced model).

MATERIALS AND METHODS

Plant material

The plant material, *Cassia fistula* was collected from Chennai, South India and it was authenticated by Dr. Jayaraman, Director, Plant Anatomy Research Centre, Chennai.

Extraction

About 500 gm of powder was extracted with alcohol and water by maceration method for 48 hours. The extracts were filtered and distilled off and the final traces of solvent were removed under vacuum and their percentage yields were 7.58% and 12.05% respectively.

Phytochemical screening

In order to determine the presence of various phytoconstituents, a preliminary phytochemical study (color reactions) with plant extracts viz. alcohol and aqueous extracts were performed[8].

Animals

Healthy Wistar rats of either sex (150-180g) with no prior drug treatment were used for the present studies obtained from Institutional Animal Breeding House, Vels School of pharmaceutical sciences, Chennai-117. Animals were housed in plastic cages at an ambient temperature (25±2°C) and relative humidity of 45-55%. A 12:12 hr light-dark cycle was maintained during the experiments. They were fed with balanced rodent pellet diet from Poultry Research Station, Nandanam, Chennai-35 and water *ad libitum* throughout the experimental period. Animals were acclimatized to their environment for at least one week before experimentation.

Acute toxicity studies

The acute toxicity test of the extracts was determined according to the OECD (Organisation for Economic Co-operation and development) guidelines 423. The animals of various groups containing five animals in

TABLE 1 : Hypoglycemic activity of *Cassia fistula* bark extracts in normal rats

Treatment	Dose mg/kg	Blood glucose concentration (mg/dl)				
		0 h	½ h	1 h	2 h	3 h
Normal control	-	79.54±0.75	79.04±0.36	78.87±0.90	80.03±1.63	78.38±1.70
Glibenclamide	5	79.20±2.02	48.30±1.43**	39.60±1.63**	36.40±1.39**	40.90±0.86**
Alcoholic extract	400	80.03±2.44	72.53±3.15**	68.93±2.10**	62.73±2.66**	65.98±1.34**
Aqueous extract	400	82.20±2.15	71.72±3.05**	66.72±1.70**	60.36±1.52**	65.02±1.20**

Values are mean±SEM of 5 replicates. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's 't' test (n=6). The values are ***P<0.001, **P<0.01, when compared against control

TABLE 2 : Effect of *Cassia fistula* bark extracts on biochemical parameters in diabetic rats

Parameters	Experimental groups				
	Normal control	Diabetic control	Glibenclamide (5mg/kg)	Ethanolic extract (400mg/kg)	Aqueous extract (400mg/kg)
Blood glucose	79.54±0.75**	302.41±1.05	67.74±1.20**	76.37±2.15**	74.82±0.78**
S. urea	30.45±1.39**	176.00±4.61	32.73±3.90**	62.54±4.09**	60.39±2.70**
S. creatinine	0.46±0.02**	1.56±0.20	0.44±0.06**	0.60±0.05**	0.58±0.01**
S. cholesterol	37.32±4.62**	86.30±5.20	36.84±1.47**	46.20±2.82**	44.28±0.82**
S. triglyceride	32.40±2.84**	97.38±3.92	34.00±0.72**	69.48±2.91**	68.90±4.71**
HDL	24.80±1.26**	10.68±1.08	25.83±2.86**	18.90±1.02*	19.60±2.89*
LDL	22.84±4.81**	57.82±2.09	22.46±3.84**	37.82±0.92**	38.00±2.89**
Haemoglobin	11.60±0.44	7.90±4.75	10.82±5.90	9.72±0.42	9.86±3.47
Gly.haemoglobin	1.86±0.25**	5.72±0.37	2.60±0.20**	4.10±0.34*	3.62±0.64**

Values are mean±SEM of 5 replicates. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's't' test (n=6). The values are **P<0.01, *P<0.05 when compared against control

each group were treated with the starting dose of 2000mg/kg of the test samples and monitored for 14 days, for mortality and general behavior. No death was observed till the end of the study[9]. The test samples were found to be safe up to the dose of 2000mg/kg and from the results; 400 mg/kg dose was chosen as the therapeutic dose for further experimentation.

Hypoglycemic activity

The animals were classified in to four groups (n=5). They were fasted overnight. Group 1 was kept as control, and was given a single dose of 0.5 ml/100 g of the vehicle; group 2 was treated with glibenclamide (5mg/kg) as the hypoglycemic reference drug. Groups 3 and 4 were treated with ethanolic and aqueous extracts at the dose level of 400mg/kg (p.o.), as mentioned in the TABLE 1. Blood samples were collected from tail tip at 0 (before oral administration), 0.5, 1, 2 and 3 h after administration[10]. The blood sugar level was measured using Accu-chek Active™ Test strips in Accu-chek Active™ Test meter.

Antidiabetic activity

Alloxan induced diabetic model was selected to confirm the effectiveness of the active antihyperglycemic extract in experimental diabetic conditions. Diabetes was induced in rats by injecting 120mg/kg of alloxan monohydrate intraperitoneally in 0.9% w/v NaCl to overnight-fasted rats. The rats were then kept for the next 24 h on 10% glucose solution bottles, in their cages, to prevent hyperglycemia. After 72 h of injection, fasting blood glucose level was measured. The animals that did not develop more than 300 mg/dl glucose levels were rejected[11]. The selected diabetic animals were divided into four groups (n=5) and one more group of normal non-alloxanised animals was also added to the study. Group 1 was kept as normal control (non-alloxanised rats), which received a single dose of 0.5 ml/100 g of the vehicle; group 2 was kept as negative control, alloxan induced and received a single dose of 0.5ml/100 g of the vehicle; group 3 , diabetic induced, was treated with glibenclamide (5mg/kg) as the reference drug. Groups 4 and 5, diabetic induced were treated with alcoholic extract and aqueous extract 400mg/kg. The treatment was continued for seven consecutive days (p.o.). At the end of the 7th day, the rats were fasted for 16 h and blood parameters were determined.

Collection of blood and estimation of biochemical parameters

The blood sugar level was measured using Accu-chek Active™ Test strips in Accu-chek Active™ Test meter by collecting the blood from rat tail vein. For other plasma profiles, blood was collected from retro-orbital plexus of the rats, under light ether anesthesia, using capillary tubes into eppendorf tubes containing heparin. The plasma was separated by centrifugation (5 min, 5000rpm) and was analysed for lipid profiles (serum cholesterol, serum triglyceride, HDL cholesterol, and LDL cholesterol), serum creatinine, serum urea, hemoglobin and glylosylated hemoglobin. The plasma profiles were measured by standard enzymatic methods, with an automatic analyzer and the results were presented in TABLE 2 (Tulp, Goa, Model No. Evaluation 300) and glycosylated hemoglobin by colorimetric method[12].

Statistical analysis

The values are expressed as mean±SEM. The results were analyzed for statistical significance using one-way ANOVA, followed by Dunnet's test. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The alcoholic extract showed the presence of steroids, carbohydrates, tannins, flavonoids and anthraquinone glycosides and aqueous extract contained steroids, saponins, carbohydrates, tannins, flavonoids and anthraquinone glycosides.

The alcoholic and aqueous extracts were subjected to hypoglycemic studies at a dose level of 400mg/kg and the results were given in TABLE 1. Both the tested extracts of *Cassia fistula* showed significant hypoglycemic activity in fasted normal rats.

The basal blood glucose levels of all the groups were statistically not different from each other. After seven days, the values of blood glucose were decreased in all the treated groups and the diabetic rats

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showed a slight increase in blood glucose level. Sustained reduction in hyperglycemia in diabetes mellitus will decrease the risk of developing micro-and macrovascular complications. The administration of plant extract and glibenclamide to diabetic rats restored the level of blood glucose significantly (P<0.01). Glibenclamide is reported to enhance the activity of Beta cells of the pancreas resulting in secretion of larger amount of insulin which in turn brings down blood glucose level[13] so the mechanism behind the hypoglycemic and antidiabetic effect of extracts suggests an insulin-like effect[14].

In uncontrolled or poorly controlled diabetes, there is an increased glycosylation of a number of proteins including hemoglobin. Therefore, the total haemoglobin level is decreased and glycosylated haemoglobin is increased in alloxan diabetic rats[15]. The administration of plant extract and glibenclamide to diabetic rats restored the changes in the level of glycosylated haemoglobin to near normal levels (P<0.01 and P<0.05). But, both the tested extract did not show significant effect in increasing the total haemoglobin content.

The levels of serum lipids are usually elevated in diabetes mellitus which represents a risk factor for coronary heart disease. This abnormally high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots, mainly due to the actions of insulin. Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However in diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia[16]. Alloxan induced diabetic rats showed significant hypercholesterolemia as compared with control. Treatment with plant extract showed significant decrease in cholesterol levels (P<0.01); at the same time, it showed an increase in HDL-c (P<0.05). Hypercholesterolemia was associated with hypertriglyceridemia which was also significantly prevented by treatment with the plant extract (P<0.01). Treatment with plant extract of *Cassia fistula* significantly decreased creatinine and urea levels (P<0.01) which are considered significant markers of renal dysfunction[17]. Thus the tested extract (ethanolic and aqueous) was found to be effective in alleviating experimental diabetes and diabetes related complications (TABLE 2).

We conclude that the plant has shown potential activity in decreasing the serum glucose level and other complications associated with experimental diabetes. This research supports the inclusion of this plant in traditional antidiabetic preparations.

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