

Original Research Article

Assessment of anti-diabetic activity of *Cassia sophera* (Caesalpiaceae)

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Abstract

Purpose: To examine the ethnomedicinal claims regarding the antidiabetic uses of *Cassia sophera* L. (Caesalpiaceae) using an alloxan-induced diabetes model.

Methods: The methanol extracts of leaves (CSLM) and roots (CSRM) of *C. sophera* were evaluated for hypoglycemic and anti-diabetic activities in normal and alloxan-induced diabetic rats. Alloxan (120 mg/kg, i.p.) was administered to induce diabetes in rats. A dose regime of 50, 100 and 200 mg/kg of CSLM and CSRM was given orally to the respective groups (n = 6). Blank group consisted of normal animals maintained on saline. The diabetic untreated group served as negative control while the group that received glibenclamide (5 mg/kg) was positive control.

Results: A significant ($p < 0.05$) lowering of fasting blood glucose level FBG in oral glucose tolerance test (OGTT) in normal rats was observed after daily administration of the extract for 7 days. Both extracts demonstrated decreased ($p < 0.05$) BGL with significantly ($p < 0.05$) improved glucose tolerance and body weight at the end of 4th, 7th and 14th day in extract-treated diabetic rats when compared with negative control and normal untreated group, respectively. In normoglycemic rats, CSLM and CSRM showed dose-dependent decrease in BGL.

Conclusion: These results suggest that both extracts possess significant blood glucose lowering activity in normal as well as in diabetic rats.

Keywords: *Cassia sophera*, Hypoglycemic activity, Anti-diabetic activity, Alloxan, Weight loss

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INTRODUCTION

Diabetes mellitus, at present, affects approximately 220 million people worldwide and this number is highly expected to increase in double proportions by 2030. At present, diabetes mellitus and its associated complications are reported to cause the death of six people every

minute [1]. Impaired glucose tolerance (postprandial and fasting hyperglycemia) and variations in lipid and protein metabolism associated with absolute or relative insulin resistance and pancreatic β -cell dysfunction are typical features of Type 2 diabetes. Therefore, haemostatic balance of glucose is needed to minimize the complications associated with the

disease [2].

Increased insulin secretion by β -cells, enhanced sensitivity of organs to insulin and reduced postprandial glucose absorption are the exemplary approaches, which are in practice to deal with risk factors of diabetes mellitus [3]. However, clinically used anti-diabetic agents such as glibenclamide produce serious side effects (cholestatic jaundice, anemias, renal failure etc.) [4]. Therefore, effective and safer anti-diabetic agents that can cope with complications associated with diabetes mellitus are still needed [5]. Plants offer an easily accessible and cost-effective source for the discovery of such therapeutic agents with lesser side effects [6].

Cassia sophera L. (Family Caesalpiniaceae) is an annual shrub found throughout tropical and sub-tropical regions in Pakistan, India and China. It is 1 - 3 m tall with compound leaves consisting of 4 - 12 pairs of leaflets. The ethnomedicinal reputation of this plant includes its use in treatment of diabetes, rheumatic disorders, constipation, bronchitis, asthma and liver diseases [7]. It is considered to possess anticancer and anti-inflammatory properties. Flavonoids, anthraquinones, saponins, sterols, tannins and terpenoids have been isolated from root, stem bark and whole plant ethanol extracts of *C. sophera* [5,8]. The aim of the present study was to evaluate the anti-diabetic activities of the leaf and root extracts of *C. sophera* in order to ascertain its ethnomedicinal use.

EXPERIMENTAL

Plant material

The plant material was collected from Sargodha District, Pakistan in December 2012 and authenticated by Prof Dr Altaf Ahmad Dasti, Director, Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan. A specimen bearing voucher no. SWT-366 was preserved in the herbarium of the institution. The plant material was air-dried under shade for 15 days at room temperature. The dried plant material was powdered using grinder and stored in air-tight jars under refrigeration for further research.

Extraction

The maceration of powdered leaves and roots (500 g each) was done in methanol for a period of 24 h separately and then filtered. The procedure was repeated thrice using 1.0 L methanol in each step. The filtrates from each

step were combined and concentrated using rotavapor (Buschi, Switzerland) at 35 °C. The roots yielded crude methanol extract 25.3 g (5.06 % of dry weight). While the yield of leaf methanol extract was 40.5 g (8.8 % of dry weight)

Animals

Adult Wistar rats with body weight range of 180 – 220 g were used in the experiments. The animals were kept in the animal house of Department of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan. A standard room temperature of 25 ± 1 °C; relative humidity of 45 – 55% and a 12 h light/dark cycle for 7 days with free access to food and water *ad libitum* under hygienic conditions were maintained. Before start of experiments, animals were acclimatized to laboratory conditions for 48 h. The Institutional Animal Ethical Committee of Department of Pharmacy, B. Z. U. Multan granted approval for the animal experiments (no. IAEC/Approval/01/2014/Pharm) as per OECD guidelines 2006 [9]

Assessment of hypoglycaemic activity in normal rats

Animals were divided into eight groups (n = 6). Groups 1 received distilled water. Groups 2, 3 and 4 received CSLM at doses of 50, 100 and 200 mg/kg, p.o. daily. Groups 5, 6 and 7 were administered 50, 100 and 200 mg/kg doses of CSRSM orally. Group 8 received Glibenclamide (5 mg/kg, p.o.) as a standard drug. Treatment was done daily for 7 days. Animals in all the groups received glucose (2 g/kg, p.o.), 30 min after the extract or standard drug administration in order to estimate oral glucose tolerance. Blood samples were collected from the tail vein prior to (0 h) and at 1, 2, 4 h after glucose administration on the 0 day and on the 4th and 7th days after 16 h of overnight fasting for glucose level estimation during 7 days of treatment period [1].

Assessments of anti-diabetic activity in alloxan-induced diabetic rats

A fresh solution of alloxan monohydrate was prepared and subsequently administered to rats at a dose of 120 mg/kg body wt. In order to prevent hypoglycaemia following alloxan injection, the rats were maintained on a glucose solution (5 % w/v) for next 24 h. The rats showing persistent hyperglycaemia for next 7 days within the range of 290 to 400 mg/dL (fasting blood glucose level) were selected. Such animals were divided into nine groups namely Group 1 (Normal untreated), Group 2 (Diabetic untreated), Group 3 (CSLM 50 mg/kg, p.o.),

Group 4 (CSLM 100 mg/kg, p.o.), Group 5 (CSLM 200 mg/kg, p.o.), Group 6 (CSRM 50 mg/kg, p.o.), Group 7 (CSRM 100 mg/kg, p.o.), Group 8 (CSRM extract 200 mg/kg, p.o.) and Group 9 (Glibenclamide 5 mg/kg, p.o.).

Following administration of extracts/glibenclamide daily for 14 days to the respective groups, blood samples were collected by retro-orbital puncture at 0, 1, 2, and 4 h on the 4th, 7th and 14th day for estimation of blood glucose level. Oral glucose tolerance test (OGTT) was performed on day 14 by orally administering glucose at a dose of 2 g/kg b.w. 30 minutes after the administration of extracts/glibenclamide. Measurement of body weights was also carried out on the 0, 4th, 7th, and 14th day an hour following treatment with the extracts/glibenclamide in order to calculate percentage change of the body weight [1].

Statistical analysis

The data are expressed as mean \pm SEM and were analyzed using one-way ANOVA followed by *post hoc* Duncan's multiple range tests using SPSS software, version 15 (SPSS Inc, Chicago, IL, USA). $P < 0.05$ was considered statistically significant. $P < 0.05$ was considered statistically significant.

RESULTS

The methanol extract of roots (CSRM) and leaves (CSLM) significantly ($p < 0.05$) decreased fasting blood glucose level (FBG) in normal rats at 1, 2 and 4 h after treatment with 100 and 200 mg/kg of each extract. The dose of 200 mg/kg caused greater reduction as compared to that of 100 mg/kg dose. However, 50 mg/kg of each extract failed to produce hypoglycemia. This leads to an assumption that the extracts caused hypoglycemia in normal rats more or less in a dose dependent manner. The hypoglycemic effect was more pronounced with CSRM after

single dose administration. Repeated dose administration of CSRM and CSLM produced a significant ($p < 0.05$) reduction of FBG on 4th, 7th and 14th day with all doses, indicating that both extracts can produce significant hypoglycemia on repeated administration. Both extracts at higher dose level i.e. 200 mg/kg, significantly ($p < 0.05$) reduced FBG level after single and repeated dose administration which is comparable with the FBG reduction produced by the positive control, glibenclamide (5 mg/kg) (Table 1).

The effects of CSRM and CSLM on blood glucose level of glucose loaded rats in oral glucose tolerance test after 7 days pretreatment with test extracts was also studied. The blood glucose level (BGL) of rats pretreated for 7 days was significantly ($p < 0.05$) reduced with CSRM and CSLM at 1 and 2 h at all doses as compared with normal control. Glibenclamide (5 mg/kg) significantly ($p < 0.05$) suppressed the BGL rise at all sampling intervals (Table 2).

The significant ($p < 0.05$) reduction of the elevated BGL was observed on repeated administration of the CSLM and CSRM in normoglycemic rats when compared with the control groups. The results of the oral glucose tolerance test showed that both extracts produced significant ($p < 0.05$) reduction of the blood glucose levels at all dose levels on 1 and 2 h intervals in normoglycemic rat. Intravenous injection of alloxan (120 mg/kg) resulted in raised BGL in rats with a range of 290-310 mg/dL after 5 days. A significant reduction ($p < 0.05$) in BGL was observed following single dose administration of CSRM and CSLM (100 and 200 mg/kg) at 1, 2 and 4 h. However, Glibenclamide caused pronounced reduction in BGL at 1 and 2 h after single dose in alloxan-induced diabetic rats. Repetition of dose regimen with CSRM and CSLM (50, 100 and 200 mg/kg) resulted in significant ($p < 0.05$) and progressive decrease in the BGL over a period of two weeks.

Table 1: Hypoglycemic activity of *C. sophera* in normal rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dL)						
		0 h	1 h	2 h	4 h	4 th day	7 th day	14 th day
Normal untreated	-	99.38 \pm 2.1	98.84 \pm 1.2	99.27 \pm 1.88	98.81 \pm 2.1	99.16 \pm 1.96	98.52 \pm 2.17	98.82 \pm 1.8
CSLM	50	98.99 \pm 1.98	98.31 \pm 2.01	98.12 \pm 1.93	97.93 \pm 2.34	93.87 \pm 1.98*	89.31 \pm 2.62*	84.99 \pm 1.81*
CSLM	100	99.45 \pm 1.83	95.31 \pm 2.34*	92.98 \pm 1.83*	86.3 \pm 1.79*	80.57 \pm 2.04*	74.34 \pm 3.2*	69.89 \pm 2.89*
CSLM	200	98.87 \pm 1.9	93.11 \pm 1.68*	90.47 \pm 2.27*	83.05 \pm 1.94*	74.69 \pm 1.79*	68.34 \pm 2.7*	64.29 \pm 1.29*
CSRM	50	97.94 \pm 2.7	97.31 \pm 1.82	97.13 \pm 1.89	96.73 \pm 2.04	92.45 \pm 2.11*	87.93 \pm 1.89*	81.16 \pm 2.89*
CSRM	100	99.19 \pm 2.19	94.83 \pm 2.19*	90.65 \pm 2.07*	83.83 \pm 1.93*	78.87 \pm 1.98*	72.34 \pm 2.19*	68.79 \pm 1.95*
CSRM	200	98.59 \pm 1.93	92.61 \pm 2.85*	89.33 \pm 1.88*	77.09 \pm 1.82*	69.3 \pm 2.13*	63.47 \pm 2.13*	59.38 \pm 2.09*
Glibenclamide	5	99.69 \pm 2.08	91.86 \pm 1.64*	85.53 \pm 1.58*	73.89 \pm 1.67*	64.08 \pm 2.3*	58.39 \pm 1.62*	53.23 \pm 2.38*

Table 2: Effect of *C. sophera* on BGL of glucose loaded rats (OGTT) after 7 days pretreatment

Treatment	Dose (mg/kg)	Blood glucose level (mg/dl)			
		0 h	1 h	2 h	4 h
Normal untreated	-	99.88±2.4	149.88±4.21	124.34±2.67	102.94±2.45
CSLM	50	88.39±1.92	136.54±2.34*	114.54±2.03*	103.07±2.21
CSLM	100	74.67±3.45	121.56±2.07*	101.59±1.92*	97.16±1.45
CSLM	200	67.53±2.3	116.39±2.48*	92.24±2.72*	95.2±1.96
CSRM	50	86.34±1.87	132.97±2.5*	109.92±3.15*	103.47±2.6
CSRM	100	71.19±2.21	116.91±3.21*	99.5±2.37*	95.81±1.59
CSRM	200	63.56±1.95	110.09±1.89*	88.53±2.63*	91.97±2.19*
Glibenclamide	5	58.48±1.59	106.96±2.61*	83.15±1.98*	87.27±1.9*

Data are expressed as mean ± SEM (n = 6); * $p < 0.05$ versus the normal untreated control group

Animals treated with the methanol extract of roots and leaves at 100 and 200 mg/kg exhibited significant decrease ($p < 0.05$) in BGL on 4th, 7th and 14th day of treatment when compared to other groups of animals. The 50 mg/kg dose for each extract showed significant reduction ($p < 0.05$) of BGL on 7th day and onwards. The results showed that CSRM was more potent compared to CSLM as it caused more reduction of BGL in diabetic animals. The results revealed the significant anti-hyperglycemic activity of *C. sophera* in alloxan-induced diabetic rats (Table 3).

Effects of methanol extracts of leaves and roots of *C. sophera* on blood glucose level of glucose loaded diabetic rats in oral glucose tolerance test on 14th day following treatment with test extracts were studied. Oral treatment with the CSRM and CSLM at all dose levels for 2 weeks had exhibited significantly improved results in OGTT as compared to diabetic untreated group (Table 4). Alloxan (120 mg/kg, i.v.) had resulted in significant reduction ($p < 0.05$) of the body weight at the end of 7th and 14th day as compared to normal untreated group. Repeated dose treatment with Glibenclamide (5 mg/kg) caused prevention of body weight loss on 4th, 7th and 14th day in alloxan-induced diabetic rats.

Table 3: Effect of *C. sophera* on blood glucose level in alloxan-induced diabetic rats

Treatment	Dose (mg/kg)	Blood glucose level (mg/dL)						
		0 h	1 h	2 h	4 h	4 th day	7 th day	14 th day
Normal untreated	-	99.48±2.2*	98.78±1.82*	100.21±1.81*	99.21±1.9*	99.76±2.1*	99.25±2.01*	98.99±1.93*
Diabetic untreated	-	300.34±4.32	300.27±3.78	299.12±4.21	297.83±5.01	299.53±3.96	297.88±4.82	299.2±4.31
CSLM	50	298.39±2.91	295.64±4.72	293.21±3.17	291.93±4.57	285.21±5.01	260.19±4.01*	221.43±4.51*
CSLM	100	302.85±3.18	281.18±3.49*	269.01±3.81*	228.42±4.12*	176.14±3.49*	158.36±4.28*	134.15±3.74*
CSLM	200	300.68±3.21	275.48±3.76*	256.31±4.1*	208.28±3.59*	156.02±2.7*	133.27±4.2*	110±3.59*
CSRM	50	297.79±4.01	294.38±3.1	292.91±4.29*	291.11±4.18	284.49±5.34	251.11±4.36*	209.2±3.9*
CSRM	100	296.94±3.71	278.48±4.15*	264.61±3.92*	219.13±3.61*	163.74±3.82*	148.71±3.96*	126.9±4.26*
CSRM	200	302.71±3.58	272.62±3.36*	244.19±4.5*	201.37±3.76*	146.19±3.12*	125.31±4.27*	100.3±4.19*
Glibenclamide	5	301.23±3.72	267.51±4.31*	228.82±4.21*	189.05±3.91*	132.7±3.56*	108.35±2.73*	91.39±3.08*

Values are expressed as mean ± SEM (n = 6); * $p < 0.05$ versus diabetic untreated control group

Table 4: Effect of *C. sophera* on BGL of glucose loaded diabetic rats (OGTT) on 14th day

Treatment	Dose (mg/kg)	Blood glucose level (mg/dL)		
		1 h	2 h	4 h
Normal untreated	-	148.15±4.28	122.59±3.6	103.71±2.9
Diabetic untreated	-	451.29±5.34	370.56±4.67	310.6±4.43
CSLM	50	254.89±2.9*	236.13±3.24*	218.48±3.1*
CSLM	100	176.55±3.09*	134.32±3.04*	103.71±2.93*
CSLM	200	153.8±3.38*	120.16±3.41*	94.61±2.63*
CSRM	50	240.78±4.83*	358.01±4.25*	288.51±3.89*
CSRM	100	167.53±3.7*	135.19±5.39*	99.85±4.3*
CSRM	200	139.09±2.95*	112.79±3.57*	91.37±4.17*
Glibenclamide	5	129.58±1.98*	103.67±3.62*	84.78±2.75*

Values are expressed as mean ± SEM (n = 6); * $p < 0.05$ versus the normal untreated control group

Table 5: Effect of repeated dose treatment of *C. sophera* on body weight in alloxan-induced diabetic rats

Treatment	Dose (mg/kg)	Body weight (% Change from initial weight)		
		4 days	7days	14 days
Normal untreated	-	1.34±0.72	2.43±0.82	4.12±1.09
Diabetic untreated	-	-5.21±0.79	-9.31±1.29	-11.74±0.89
CSLM	50	-5.11±0.91	-8.62±0.82	-7.81±1.19*
CSLM	100	-4.62±1.29*	-7.32±0.91*	-5.81±1.93*
CSLM	200	-4.11±0.82*	-6.43±0.98*	-4.49±0.71*
CSRM	50	-5.06±1.28	-8.01±0.88*	-6.94±1.57*
CSRM	100	-4.35±0.44*	-7.03±1.46*	-5.29±0.71*
CSRM	200	-3.89±0.68*	-6.29±1.51*	-4.17±1.53*
Glibenclamide	5	-3.78±0.81*	-5.72±0.68*	-3.19±0.79*

Values are expressed as mean ± S.E.M (n = 6); * $p < 0.05$ versus normal untreated control group

These results show that CSRM (100 and 200 mg/kg) and CSLM at 200 mg/kg significantly prevented ($p < 0.05$) the reduction in body weight after the 4th day onwards, whereas CSRM at 50 mg/kg was only able to significantly inhibit ($p < 0.05$) the body weight loss only after the 7th day. The CSLM (50 mg/kg) significantly inhibited ($p < 0.05$) the body weight loss on the 14th day (Table 5).

DISCUSSION

In order to determine the anti-diabetic effects of the methanol extracts of leaves and roots of *C. sophera*; hypoglycemic effects on normal rats, anti-diabetic activity in alloxan-induced diabetic rats and anti-hyperglycemic actions both in normal and alloxan-induced diabetic rats following oral glucose challenge were evaluated. The effects of methanol extracts of leaves and roots of *C. sophera* on weight loss in diabetic rats were also assessed.

The results of OGTT in normal rats could be correlated with the ability of the extracts to probably enhance the secretion of insulin in the likely manner of sulfonylureas and inhibit α -glucosidases present in the border brush of small intestine [1 & 10]. Enhanced tissue uptake of blood glucose induced by *C. sophera* might also be taken into consideration as an alternate possibility. Furthermore, both the extracts exerted prominent glucose lowering effect when compared to normal untreated rats which leads to the possibility of an increased peripheral glucose consumption owing to insulin like effect, delayed insulin catabolism and reduced/inhibited glucose reabsorption by the kidneys [3-5].

Alloxan destroys β -cells of pancreatic islets of Langerhans which results in reduced insulin secretion thereby inducing hyperglycaemia [10]. The alloxan-induced diabetic rats exhibited persistent elevation in BGL which is the typical

sign of diabetes mellitus. Both extracts of *C. sophera* significantly decreased the BGL in alloxan-induced diabetic rats on single dose administration, which infers that both extracts are effective. In alloxan-induced diabetic rats, impaired glucose tolerance is achieved because of lack of insulin which is attained by destructing the β -cells of pancreatic islets of Langerhans ultimately leading to type I diabetes [11].

The study indicates that CSLM and CSRM can bring about improvements in glucose tolerance in alloxan-induced diabetic rats, which reveals that insulin mimetic activity or improved glucose utilization mechanism may be causative factors. The possibility of insulin secretion from β -cells or its release from bound form cannot be ruled out as responsible mechanisms of the extract in causing anti-hyperglycemia in alloxan-induced diabetic animals [12]. Increased secretion of insulin from β -cells of islets of Langerhans in mild alloxan-induced diabetic animals (some of the β -cells still surviving) caused by sulfonylureas, is an established fact.

Like glibenclamide which reduced BGL in mild hyperglycaemic animals, CSLM and CSRM produced normalization of BGL compared to diabetic untreated control group. The possible mechanism producing this effect may be CSLM and CSRM-induced release of insulin from some surviving β -cells [13]. This assumption is supported by the fact that some herbal preparations can stimulate β -cells regeneration and peripheral glucose consumption in alloxan- and streptozotocin-induced diabetic rats [4,14]. The diabetogenic action of alloxan is mediated through the damaging action of reactive oxygen species on β -cells. The medicinal plants have been shown to exert anti-diabetic activity due to antioxidant action of secondary metabolites such as flavonoids, isoflavonoids, coumarins, tannins, phenolic acids and triterpenoids [4,15]. Thus, the scavenging of alloxan-induced free radicals by

these bioactive phytoconstituents might have resulted in enhanced β -cells regeneration [3,15] ultimately leading to anti-hyperglycemic activity of *C. sophera*.

Weight loss is a serious complication of diabetes mellitus, which occurs due to the failure of insulin to convert glucose into glycogen. The other reason is inhibition of lipolysis due to insulin unavailability resulting from pancreatic β -cells destruction. These factors lead towards animal weight loss due to muscle wasting and ultimately death. A substantial improvement in body weights of alloxan-induced diabetic animals treated with CSRM and CSLM over the whole span of the study was observed.

Weight loss reduction could be due to improved transportation of glucose into peripheral tissue because of decreased insulin resistance, enhanced lipid and protein metabolism [15]. These could be the implications of direct effects of *C. sophera* components on glucose transportation, lipolysis and protein metabolism. The results of blood glucose level, oral glucose tolerance and body weight were in good correlation with each other and indicated the beneficial effects of *C. sophera* on alloxan-induced diabetic model.

CONCLUSION

The findings of this study indicate that *C. sophera* extract possesses antidiabetic activity. This lends some support for the traditional use of this herb in the management of diabetes. However, further studies are required to determine its toxicological and safety profile.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this study.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this

article will be borne by the authors. The study was conceived and designed by Prof Dr Bashir Ahmad, plant was collected by Khizar Abbas and dried and pulverized by Khuram Ashfaq. Experimental work was conducted by Muhammad Naeem Qaisar and Sajid Nawaz Hussain. Data handling and statistics application was done by Muhammad Uzair. Manuscript was written by Sajid Nawaz Hussain and Muhammad Naeem Qaisar. All authors read and approved the manuscript for publication.

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