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Antidiabetic and anti-lipemic effects of *Cassia siamea* leaves extract in streptozotocin induced diabetic rats

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ABSTRACT

Objective: To investigate the antidiabetic and anti-lipemic effects of *Cassia siamea* methanolic leaves extract. **Methods:** The antidiabetic study was performed by measuring blood glucose level with elegance glucometer at weekly intervals *i.e.* 0, 7, 14 and 21 in normal and streptozotocin induced diabetic rats. Total cholesterol, triglyceride and HDL-cholesterol were determined in normal and streptozotocin induces diabetic rats by autoanalyser. Glibenclamide was used as a reference drug at a dose of 10 mg/kg. **Results:** After the oral administration of extracts at doses of 250 mg/kg and 500 mg/kg for three weeks, blood glucose levels and body weights were significantly improved ($P<0.01$). Daily oral treatment with the extract also resulted in significantly reduction of serum cholesterol and triglycerides. HDL-cholesterol level was found to be improved to ($P<0.01$). **Conclusions:** The *Cassia siamea* leaf extract is useful in controlling blood glucose level as well as improving lipid metabolism and body weight in rats with induced diabetes.

1. Introduction

Diabetes is a metabolic disorder characterized by deficiency in production of insulin by pancreas, or by ineffectiveness of the produced insulin[1]. Synthetic antidiabetic agents may induce serious side effects thus are not suitable for use during pregnancy. In view of the adverse effects associated with the synthetic drugs, conventional antidiabetic plants exploration has aroused wide interest among researchers[2]. There are more then 1 200 plants species worldwide that are used in the treatment of diabetes mellitus and a substantial number of plants have shown effective hypoglycemic activity after laboratory testing[3]. Furthermore, after the recommendation made by WHO on diabetes mellitus, investigations on hypoglycemic agents from medicinal plants have become more important [4]. Also, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine since ancient times.

Cassia siamea Lam. (Fabaceae) (*C. siamea*) is commonly known as 'iron wood tree.' The leaves of the plant are used as purgative and roots are employed for preventing convulsions in children[5]. Flowers of *C. siamea* have antioxidant activity[6]. The stem bark has analgesic, anti-inflammatory and antiplasmodial effects[7,8]. Many tribal people have been using *C. siamea* leaves in diabetes problems. Furthermore, the plants from same genus *Cassia kleinii*, *Cassia auriculata* and *Cassia glauca* have been reported for antidiabetic activity[9-11].

There is no previous report available on antidiabetic use of the plant. Hence, we report detail studies on antidiabetic and anti-lipidemic of the methanolic extract from leaves of *C. siamea* methanolic leaves extract (CSMLE), with a view to provide scientific evidence of modern lines.

2. Materials and methods

2.1. Plant material

C. siamea leaves were collected from the campus of Kurukshetra University, Kurukshetra, India and were identified by Dr. B.D. Vashishta, Department of Botany, Kurukshetra University, Kurukshetra, India. A voucher specimen of the plant is preserved in the herbarium of the Faculty of Pharmaceutical Sciences, Kurukshetra University (No. IPS/KUK/CS/2009).

2.2. Extraction

The leaves were dried under shade and powdered to coarse particles. The powdered material was defatted with petroleum ether (60-80 ℃) in a Soxhlet extraction apparatus

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and further extraction with methanol was made. The extract was dried at 45 ℃ in rotary evaporator to produce a semisolid mass and stored in airtight containers in refrigerator below 10 ℃.

2.3. Chemicals

Streptozotocin was purchased from Sigma-Aldrich, India. The streptozotocin solution was prepared by freshly dissolving in citrate buffer (0.01 M, pH 4.5). Total cholesterol, high density lipoprotein (HDL)-cholesterol and triglyceride (TC) standard kits were purchased from Erba diagnostics Mannheim Gambh, Germany. All reagents used in study were analytical grade.

2.4. Animals

Wistar rat of either sex, weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of (22 ±2) ℃ and at 45%-55% relative humidity for 12 h under dark and light cycle. They were fed with a standard pellet rats diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. All the studies were conducted in accordance with the Animal Ethical Committee of the University.

2.5. Induction of diabetes

Rats were made diabetic by a single dose of streptozotocin (60 mg/kg body weight i.p.). The blood glucose level was checked before and 72 h after streptozotocin injection to confirm the development of diabetes. The diabetic animals were stabilized for five days, on the 6th day (day 0) experiment was started. Only those animals which showed blood glucose levels >250 mg/dL were separated and used for the study.

2.6. Experimental design

All the diabetic animals were randomly divided into five groups with six animals in each group. They were treated once a day for 21 days as follows:

Group I (Normal healthy control): given only vehicle (Tween 80, 1% v/v), Group II served as diabetic control: received only vehicle, Group III diabetic rats: received CSMLE 250 mg/kg.b.w., Group IV diabetic rats: received CSMLE 500 mg/kg.b.w, Group V diabetic rats: received (Glibenclamide 10 mg/kg.b.w.).

Blood glucose was measured with elegance glucometer (CT-X10, Convergent Technologies, Germany) at weekly intervals i.e. 0, 7, 14 and 21 day after daily administration of extract orally.

2.7. Anti-lipemic activity

On day 21, blood was collected by retro-orbital puncture under mild ether anesthesia from rats. Total cholesterol and triglyceride were determined by the method of Rifai *et al*[12]. HDL-cholesterol was also evaluated in normal and streptozotocin induces diabetic rats by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits by methods of Burstein *et al*[13].

2.8. Statistical analysis

All the data were expressed as mean±SEM. Statistical analysis was carried using Student's *t*-test to analyze the significance between the groups. A value of $P<0.05$ was considered to be significant.

3. Results

Daily administration of the extract for three weeks led to a dose dependent fall in blood glucose levels. Maximum effect seems to reach after 14 days of treatment and remains constant in third week. On 21st day, blood glucose (FBG) level was significantly reduced ($P<0.01$) in the diabetic rats treated with CSMLE. The antidiabetic effect of CSMLE on the blood glucose levels in diabetic rats was shown in Table 1.

There was a significant decrease ($P<0.01$) in the body weight of the diabetic controls (group II) compared with the normal controls (group I). During the weekly of observation of the flower extract-treated diabetic rats at doses of 250 mg/kg, there was significant weight gains on day 21 relative to day 0 ($P<0.05$), for rats treated with 500 mg/kg extract, the body weight almost maintained as the same (Table 1).

In the present study the total cholesterol and triglycerides was reduced in by 21 days treatment with CSMLE. HDL cholesterol level was significantly improved by treatment of CSMLE as compared to diabetic control group (Table 2).

The results of present study indicated that the methanolic leaves extract of *C. siamea* possesses significant hypoglycemic activity. It also maintained the lipid levels and body weight of rats.

4. Discussion

Streptozotocin is a nitrosurea compound produced by Streptomyces achromogenes, which specifically induces DNA strand breakage in β-cells causing diabetes mellitus. Therefore, streptozotocin has been widely employed to induce diabetes in experimental animals[14]. In this study, intraperitoneal administration of streptozotocin (60 mg/kg) effectively induced diabetes in normal rats. Diabetes is reflected by glycosuria, hyperglycaemia, polyphagia, polydipsia and body wDeight loss when compared with normal rats[15]. In diabetes the increased blood sugar levels might be due to either insulin resistance of the body cells or decreased secretion of insulin from beta cells manifest in the decreased serum insulin levels[16]. The reduction in the serum insulin levels in the streptozotocin treated rats might be attributed to the reduced secretion of the hormone which might be due to the damage of the beta cells of endocrine pancreas. The streptozotocin selectively destroys the pancreatic cells and induce hyperglycemia[17,18]. Significant reduction of blood glucose levels was observed in diabetic rats orally treated with *C. siamea* leaves 500 mg/kg ($P<0.01$).

Diabetes affects both glucose and lipid metabolism[19]. In the post prandial state elevated serum insulin increases lipoprotein lipase activity in adipose tissue and promotes fuel storage as triglycerides in normal metabolism[20]. The insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to deranged lipoprotein metabolism during diabetes[21]. The lipoprotein levels in the streptozotocin induced diabetic rats of the present study reveal a significant alter in lipoprotein metabolism. The serum total cholesterol content increased significantly in diabetic animals. The repeated administration of *C. siamea* extract for a period of 21 days resulted in a significant decrease in lipid parameter levels when compared to the diabetic control.

The levels of serum total cholesterol and tryglycerides were found to be significantly reduced in the plant extracts treated

Table 1
Effect of *C. siamea* extracts on blood glucose levels(mg/dL) and body weights(g) in diabetic rat (Mean±SEM).

Groups	Initial day		Day 7		Day 14		Day 21	
	Blood glucose level	weight	Blood glucose level	weight	Blood glucose level	weight	Blood glucose level	weight
I	115.27 ± 4.50	215.20 ± 2.30	113.34 ± 3.80	222.43 ± 4.20	112.70 ± 5.20	225.41 ± 3.60	113.82 ± 2.40	228.47 ± 3.20
II	258.41 ± 2.30	225.23 ± 3.40	294.47 ± 5.50	219.42 ± 3.80	348.70 ± 5.30	211.35 ± 2.70	402.00 ± 3.40	208.25 ± 4.30
III	280.21 ± 2.30	233.22 ± 2.10	269.40 ± 1.40*	230.32 ± 2.30*	195.22 ± 2.50*	231.13 ± 2.50*	165.42 ± 2.20*	232.85 ± 2.30*
IV	289.22 ± 1.50	230.23 ± 2.40	220.11 ± 2.30*	228.21 ± 2.80	175.24 ± 2.20**	229.32 ± 2.20*	132.45 ± 1.50**	229.92 ± 2.50*
V	274.27 ± 3.50	225.34 ± 2.70	210.72 ± 4.20**	227.34 ± 2.30*	125.41 ± 3.40*	228.78 ± 2.30*	118.53 ± 3.50**	231.25 ± 1.80*

*P<0.05, **P<0.01, When groups III IV and V compared with diabetic control *i.e.* group II.

Table 2
Effect of CSMLE on lipid profile (mg/dL).

Groups	Total cholesterol	Triglycerides	HDL cholesterol
I	87.28 ± 3.80	82.42 ± 5.16	37.32 ± 2.90
II	254.73 ± 7.60	150.52 ± 4.71	28.23 ± 2.20
III	123.32 ± 2.40*	117.32 ± 2.35*	33.24 ± 2.20*
IV	110.12 ± 1.40*	94.25 ± 3.25*	38.23 ± 1.50*
V	98.72 ± 5.30**	83.47 ± 4.50*	45.28 ± 4.80**

Data represent means ± SEM. *P<0.05, **P<0.01, When groups III IV and V compared with diabetic control *i.e.* group II.

diabetic animals. This might be due to the reduced hepatic triglyceride synthesis and or reduced The HDL increased significantly in the plant extract treated rats indicating a reversed atherogenic risk. It is not known whether the extract has a direct effect on lipids or the present hypolipidemia is achieved due to controlled hyperglycemia. It was also observed that there was also significant weight gain in CSMLE treated diabetic rats compared with untreated diabetic animals. Untill, the exact mechanism of action of reduction of blood glucose levels after administration (p.o.) of the extracts is not clear. The extracts should further be subjected to bioactivity guided drug discovery to isolate a lead compound responsible for this activity.

The *C. siamea* leaf extract is useful in controlling the blood glucose level. It also improves the lipid metabolism and body weight of rats. This could be useful for prevention or early treatment of diabetic disorders. Further studies are required to isolate, identify and characterize the active principles.

Conflict of interest statement

We declare that we have no conflict of interest.

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