International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 2, Suppl 2, 2010

Research Article

ANTI-DIABETIC ACTIVITY OF *SMILAX CHINENSIS* (L.) IN ALLOXAN INDUCED DIABETIC RATS

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Received: 02 Jan 2010, Revised and Accepted: 18 Jan 2010

ABSTRACT

Smilax chinensis L. (Liliaceae) is used in Chinese traditional medicines for treating inflammation, cancer and pain including diabetes mellitus. The anti-diabetic effects of the methanol extracts of the *Smilax chinensis* L. (MESC) on alloxan-induced diabetes were evaluated on albino wistar rats. After oral administration of each MESC singly or repeatedly to alloxan-induced diabetic rats, the blood glucose, total cholesterol (TC) and triglyceride (TG) levels were assayed. The blood glucose levels after a single oral administration of the ethanolic extract significantly reduced (p <0.01) in a time-dependent manner. Repeated oral administration of the ethanolic extract also effectively reduced the blood glucose in diabetic rats. (p < 0.01). MESC (200 & 400 mg/kg) treated groups showed significant reduction (p < 0.01) in the serum levels of Total cholesterol and Triglycerides. The results suggested that the ethanolic extract of *Smilax chinensis* L. possesses a potential hypoglycaemic effect with potential hypolipidemic effect.

Keywords: Hypoglycaemic; Hypolipidemic; *Smilax chinensis* L.; Alloxan-induced diabetic rats

INTRODUCTION

Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level [1]. This disorder occur world wide and its occurrence is increasing quickly in most of the countries [2]. Unfortunately, after the introduction of sulfonylurea and metformin about 50 years back no major lead has been obtained in this direction of finding a proper drug for diabetes[1]. This may be fulfilled by treating Diabetes mellitus with traditional medicine using as antidiabetic agents from medicinal plants.

Smilax chinensis L. (Liliaceae) is a deciduous climber with rounded leaves and red berries. The root tubes of which furnish the drug known as china root. It is found in the south Indian states namely Andhra Pradesh, Karnataka and Tamil Nadu[3]. Several species of *Smilax* are well known Chinese traditional medicines used as anti-inflammatory, antioxidants, anti-cancer and analgesic agents. The tubers of *Smilax chinensis* have been widely used in Chinese traditional medicine for treatment of diverse diseases, especially for pelvic inflammation and chronic pelvic inflammation[4-8]. In folklore medicine, it normalizes the glycemic control in diabetes[9]. Therefore the present study to investigate the antidiabetic activity of rhizomes of *Smilax chinensis* L. in Alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant collection

The Plant material of dried rhizomes of *Smilax chinensis L.* used for investigation was collected from S.V. University at Tirupathi, Chittoor (Dist.), Andhra Pradesh, India. The plant was authenticated by Dr.K.Madhava Chetty, Department of botany, S.V.University, Tirupathi. The voucher specimen of the plant was deposited at the college for further reference.

Preparation of extracts

The rhizomes of plants were dried in shade, separated and made to dry powder. It was then passed through the 40 mesh sieve. A weighed quantity (200gm) of the powder was subjected to continuous hot extraction in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of MESC was found to be 12.5% w/w.

Animals Used

Albino Wistar rats, weighing 150–200 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25 ⍰C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee. (Reg. No. IAEC/ 930/a/06/ CPCSEA).

Phytochemical Screening

The phytochemical examination of methanolic extract of *Smilax chinensis L.* rhizomes was performed by the standard methods[10].

Acute toxicity study

The acute toxicity of the methanolic extract of *Smilax chinensis L.* rhizomes was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study[11].

ANTIDIABETIC ACTIVITY

The method of Dash et al[12] was followed. The test samples were suspended in 2%v/v Tween 80 in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through p.o route.

Single dose study

In Alloxan induced diabetic rats

The acclimatized rats were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of Alloxan monohydrate in normal saline. After 1 h, the rats were provided feed *ad libitum*. The blood glucose level was checked before Alloxanisation and 24 h after Alloxanisation. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from one touch ultra, Life Scan, Johnson and Johnson, Milpitas, C.A., U.S.A.

Experimental Design

Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/dl of blood. This condition was observed at the end of 48 h after Alloxanisation. The rats were segregated into four groups of six rats in each. Group I - diabetic control and rats received only vehicle (2 ml/kg p.o) 25% Tween 80. Group II - rats received the methanolic extract of *Smilax chinensis* L. (200 mg/kg/day p.o) suspended in 2% v/v Tween 80. Group III - rats received the methanolic extract of *Smilax chinensis* L. (400 mg/kg/day p.o) suspended in 2% v/v Tween 80. Group IV – rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80. Blood glucose levels were examined after 1, 3, 5, 7 and 24 hr of administration of single dose of MESC (200 & 400 mg/kg/day p.o).

Multidose study

In Alloxan induced diabetic rats.

The selected rats were treated for 14 days with similar kind of test samples as above, but the blood glucose level was measured on initial, 3, 5, 7, and 14 days of treatment.

Estimation of serum Lipid Profile

After 14 days treatment, all the groups rats were sacrificed and estimate the Total Cholesterol, and Triglycerides level by method of Sood, 1999[13].

Histopathological study of pancreas

Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissue was carried out at the Sri Venkateswara University, Pathology Laboratory, Tirupati, Andhra Pradesh -517 502.

Statistical Analysis

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS

Phytochemical Screening

The results of preliminary phytochemical screening of the methanolic extract of *Smilax chinensis* L. revealed that presence of tannins, alkaloids, glycosides, flavonoids, carbohydrates, gums and mucilage, steroids and triterpenoids.

Effect of MESC on blood glucose level

There were observable changes in blood glucose level (BGL) and lipid profile of treated and untreated rats. Treatment of diabetic rats with the methanolic extract of *Smilax chinensis* L. and Glibenclamide significantly decreased the BGL compared to untreated diabetic rats. Dose dependent reduction in BGL, TC and TG was observed in Alloxan induced diabetic rats treated with ethanol extract of *Smilax chinensis* L.

Single dose study

After single dose of the MESC (200 or 400 mg/kg, p.o) on the Alloxan induced diabetic rats, there was a significant reduction ($P<0.01$) in BGL of the diabetic rats with in the period of acute study which was seven hours compared to the control. The effect was significant like the standard drug, Glibenclamide. MESC at the dose of 400 mg/kg body weight exhibited better BGL reduction than 200 mg/kg body weight and that produced by the standard drug, Glibenclamide 2.5mg/kg (71.42%) at the same period (Table 1).

Multidose study

During prolonged study (14 days), the MESC (200 or 400 mg/kg) produced a significant reduction ($P<0.01$) in BGL of the diabetic rats compared to control.MESC at the dose of 400 mg/kg body weight exhibited better BGL reduction than 200 mg/kg body weight and that produced by the standard drug, Glibenclamide 2.5mg/kg at the same period. (Table 2).

Serum lipid profile

Beneficial effects of MESC on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data (Table 3). Compared with the control values, the MESC (200 or 400 mg/kg) groups showed significant reduction ($P <0.01$) in the serum levels of Total cholesterol and Triglycerides.

Histopathological studies

The islets of Alloxan diabetic rats showed extensive necrotic changes followed by fibrosis and atrophy. (Fig. 1) The Alloxan diabetic rats treated with MESC 200mg/kg minimum degree of necrotic and fibrotic changes of islets of langerhans. (Fig. 2) The necrotic and fibrotic changes were not detected in the rats were treated with MESC 400mg/kg and Glibenclamide 2.5mg/kg. (Fig. 3&4)

Table 1: Effect of *Smilax chinensis* L. on blood glucose levels of Alloxan induced diabetic rats after a single dose

Groups	Drugs	Dose	Initial	1hr	3hr	5hr	7hr	24hr
Group I	Diabetic control	2% Tween 80 w/v soln p.o	287.50±1.36	284±1.57	277±1.87	286±1.39	281.5±2.03	294±1.37
Group II	Diabetic control + MESC	200 mg/kg p.o	279.67±2.26 [a]	226±1.69 [a]	210.33±1.49**[a]	167.50±2.80**[a]	129.67±2.14**[a]	101.833±1.49**[a]
Group III	Diabetic control + MESC	400 mg/kg p.o	287.83±1.83[a]	208.83±3.88**[a]	184±1**[a]	139.17±2.10**[a]	109.17±2.94**[a]	96.167±2.17**[a]
Group IV	Diabetic control + standard	Glibenclamide (2.5 mg/kg) p.o	281.67±1.94 [b]	215±1.37**[b]	141.83±1.22**[b]	129.17±1.70**[b]	98.67±2.49**[b]	84.667±1.89**[b]

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01. Significance compared with in the groups as follows: **a**. diabetic + MESC - 200 & 400 treated rats Vs. diabetic control rats. **b**. diabetic + Glibenclamide treated rats Vs. diabetic control rats.

Table 2: Effect of *Smilax chinensis* L. on blood glucose levels of Alloxan induced diabetic rats after repeated doses

Groups	Drugs	Dose	Initial	Third day	Fifth day	Seventh day	Fourteenth day
Group I	Diabetic control	2% Tween 80 w/v soln p.o	287.80±1.26	275±1.07	285±1.41	296.33±1.58	289.17±1.97
Group II	Diabetic control + MESC	200 mg/kg p.o	289.67±2.26[a]	197±1.21 [nsa]	139.5±1.20**[a]	107±2.67**[a]	102±2.42**[a]
Group III	Diabetic control + MESC	400 mg/kg p.o	297.83±1.83[a]	142.16±2.19**[a]	107.83±2.88**[a]	102±2.25**[a]	98±3.28**[a]
Group IV	Diabetic control +	Glibenclamide	281.67±1.94[b]	76.33±3.10**[b]	78.83±1.51**[b]	76.67±1.25**[b]	70.17±2.45**[b]

standard	(2.5 mg/kg) p.o	

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01. Significance compared within the groups as follows: **a**. diabetic + MESC - 200 & 400 treated rats Vs. diabetic control rats. **b**. diabetic + Glibenclamide treated rats Vs. diabetic control rats.

Table 3: Effect of *Smilax chinensis* L. on Serum Cholesterol levels of Alloxan induced diabetic rats after a prolonged treatment

Groups	Drugs	Dose	Total Cholesterol	Triglycerides
Group I	Normal Control	2% Tween 80 w/v soln p.o	158.80±2.40	74±2.07
Group II	Diabetic control	2% Tween 80 w/v soln p.o	289.5± 2.21**a	204 ± 1.88**a
Group III	Diabetic control + MESC	200 mg/kg p.o	167.66 ± 0.98*b	162.16 ± 0.87*b
Group IV	Diabetic control + MESC	400 mg/kg p.o	152 ± 1.21**b	157.66 ± 1.17**b
Group V	Diabetic control + standard	Glibenclamide (2.5 mg/kg) p.o	145.5 ± 0.99**c	138.33 ± 1.56**c

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01. Significance compared within the groups as follows: **a**. Normal control rats vs. diabetic control rats. **b**. diabetic + MESC - 200 & 400 treated rats compared with diabetic control rats. **c**. diabetic + Glibenclamide treated rats vs. diabetic control rats.

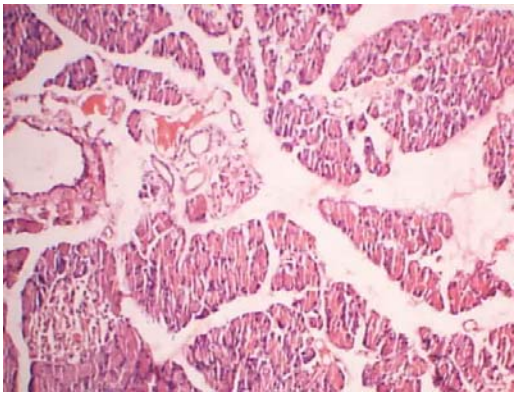

Fig. 1: Group I (Diabetic control)

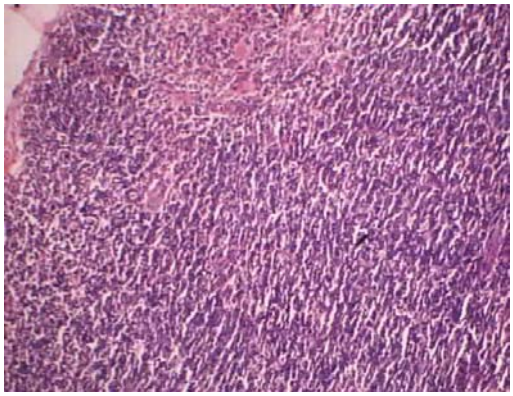

Fig. 2: Group II (Diabetic control + MESC 200)

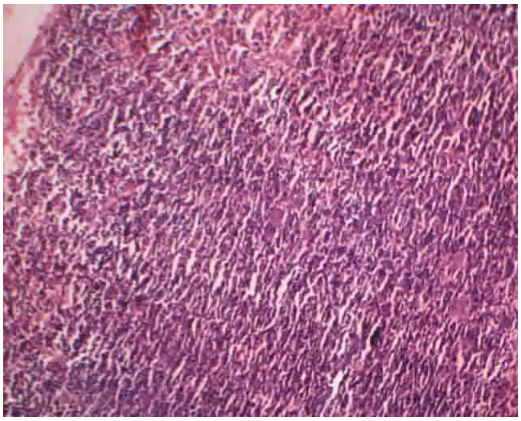

Fig. 3: Group III (Diabetic control + MESC 400)

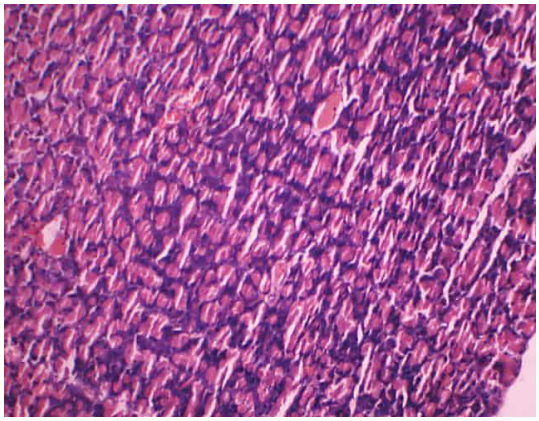

Fig. 4: Group IV(Diabetic control + standard)

DISCUSSION AND CONCLUSION

Diabetes mellitus is one of the most common chronic diseases and is associated with hyperlipidemia and co-morbidities such as obesity and hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes[14]. In order to establish a scientific basis for the utility of this plant in the treatment of diabetes, it was decided to evaluate the methanolic extract of *Smilax chinensis* L. on a single dose and multiple doses experimental design. The presence of flavanoids and triterpenoids[15] which possess hypolipidemic and antihyperglycemic properties, has been reported in literature[16,17] these compounds has been implicated in the anti diabetics activities of many plants[18].

Previous studies suggested that hyperglycemia and hyperlipidemia are the common characteristics of Alloxan-induced diabetes mellitus in experimental rats[19-21]. The maximum reduction in serum glucose levels was seen in MESC at the dose of 400 mg/kg (Table 2). hence, we could say that MESC had a beneficial effect on carbohydrate metabolism in diabetic rats. The antidiabetic activity of MESC may be it's promote insulin secretion by closure of potassium - ATP channels, membrane depolarization and stimulation of Calcium influx, an initial key step in insulin secretion. In this context, number of other plants has also been reported to have antidiabetic and insulin stimulatory effects[22,23].

In this study, we have also observed an increase in the concentration of TC and TG in alloxan induced diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus[24,25]. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose[26]. Regarding the mechanism of action MESC may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have decreased in serum cholesterol and triglycerides[27]. Most of the hypolipidemic drugs do not decrease serum TG level, but MESC lowered it significantly since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis the triglycerides[28]. And also MESC reduces the serum TG of alloxan induced diabetic rats and may prevent the progression of CHD. The total lipid profile in serum

(total cholesterol, triglycerides) of the Alloxan induced diabetes rats treated with MESC (200 or 400 mg/kg, p.o) showed significant reduction, as compared to diabetic control rats (Table 3). The strong anti-hyperglycemic effect of MESC could indirectly be related to beneficial action against the abnormal high concentration of serum lipids observed in diabetes rats.

Histopathological studies of pancreas of the diabetic rats showed necrosis, atrophy and fibrotic changes. But, the pancreas of MESC and glibenclamide treated rats showed minimal necrosis and mild atrophy and fibrotic changes.

This suggests that the herbal preparations of rhizomes of *Smilax chinensis L.* had been considered as effective economical and safe treatments for reducing the complications of lipid profile observed in diabetics with hypertriglyceremia. Additional studies are necessary to isolate and identify the active principle as well as identify possible links between Ethanol Extract of *Smilax chinensis L.* and plant's chemical composition.

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