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ANTI-DIABETIC ACTIVITY OF *SMILAX CHINENSIS L.* EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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ABSTRACT

In Indian traditional system of medicine, *Smilax chinensis* L. (Liliaceae) is prescribed for the treatment of diabetes mellitus. In the present study, the antidiabetic effect of methanol extract of *Smilax chinensis L.* leaves (MESC) was investigated in streptozotocin (STZ)-induced diabetic rats. Albino wistar rats were rendered diabetic by STZ (55 mg/kg, intraperitoneally). MESC was orally administered to STZ-induced diabetic rats at 200 and 400 mg/kg, p.o doses for 14 days to determine anti-diabetic activity. The fasting blood sugar levels and serum biochemical analysis in STZ-induced diabetic rats were investigated. Oral administration of MESC (200 & 400 mg/kg) for 14 days exhibited a significant reduction in serum glucose, total cholesterol, and triglycerides in alloxan diabetic rats. The anti-diabetic activity of the methanol extract of *Smilax chinensis L.*(MESC) were similar to those produced by glibenclamide at 600µg/kg (positive control, $p < 0.01$). The results demonstrate that MESC possesses potent anti-diabetic activity in STZ-induced diabetic rats. These very encouraging results for diabetes control by methanol extract of *Smilax chinensis L.* make the need for clinical studies in humans evident.

Keywords: *Smilax chinensis* L., STZ-induced diabetic rats, anti-diabetic, LDL, VLDL, TG, Total cholestrol.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins (Scheen, 1997). The number of people suffering from the diabetes mellitus worldwide is increasing at an alarming rate with a projected 366 million peoples likely to de diabetic by the year 2030 as against 191 million estimated in 2000 (SG Wild *et al.,* 2004). The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. In modern medicine (insulin, sulphonylureas, biguanides and thiazolidinediones), no satisfactory effective therapy is till available to cure the diabetes mellitus. In recent years, there has been renewed interest in plant medicine (Prince *et al.,* 1998; Ladeji *et al.,* 2003) for the treatment against different diseases as herbal drugs are generally out of toxic effect (Geetha *et al.,* 1994; Rao *et al*., 2003) reported from research work conducted on experimental model animal.

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 (Lee H and Lin JY, 1988). 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

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inflammation (Cui MF, 2001 ; He YL, 2002; Zheng JZ, 1996; Qiu RQ, 1997)**.** In folklore medicine, it normalizes the glycemic control in diabetes (Wong *et al.,* 2001). In previous study hypoglycemic and hypolipidemic activity of *Smilax chinensis L.* in alloxan induced diabetes in albino wistar rats was reported. Therefore the present studies to conform the anti-diabetic activity of *Smilax chinensis L.* in STZ induced diabetic rats model.

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.

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).

Experimental induction of diabetes

The animals were fasted overnight and diabetes was induced by a single intraperitoenal injection of a freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5) (Rakieten *et al.,* 1963). The animals were allowed to drink 1% glucose solution overnight to overcome the drug-induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as first day of treatment. The treatment was continued for 14 days.

Experimental design

The rats were divided into four groups comprising of six animals in each group as follows:

Group I. Control rats receiving 0.1 M citrate buffer (pH 4.5).

Group II. Diabetic control and rats received only vehicle (2 ml/kg p.o) 2% v/v Tween 80.

Group III. Diabetic rats received Methanol Extract of *Smilax chinensis L.* (200 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group IV. Diabetic rats received Methanol Extract of *Smilax chinensis L.* (400 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group V. Diabetic rats treated with glibenclamide (600 µg/kg b.w/day) suspended in 2% v/v Tween 80 orally for 14 days (Pari and Uma Maheswari, 2000).

Testing of fasting blood glucose level

Fasting blood glucose levels were measured on 0, 3, 7, and 14 days of treatment of Methanol extract of *Smilax chinensis L. leaves* supplement from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (Atkin *et al.,* 1991). The results were expressed in terms of milligram per deciliter of blood.

At the end of the experimental period, all the animals were sacrificed under light ether anesthesia. The guide line of our institutional ethical committee for this purpose was followed strictly. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used for the estimation of total cholesterol and triglycerides. Total cholesterol was estimated by the method of Parekh and Jung (1970). Triglycerides were estimated by the method of Rice (1970).

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

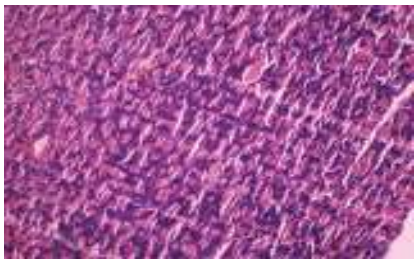
Effect of MESC on fasting blood glucose in STZ-induced diabetic rats

The effect of repeated oral administration of MESC on blood glucose levels in STZ-diabetic rats is presented in Table 1. MESC, administered at doses of 200 & 400 mg/kg to STZ-treated diabetic rats caused significant ($P < 0.01$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 14. MESC 400 mg/kg exhibited maximum glucose lowering effect in diabetic rats. Glibenclamide exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

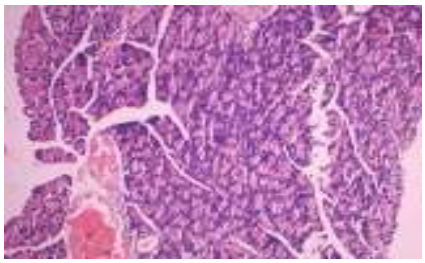
Effect of MESC on serum lipids in STZ-induced diabetic rats

MESC showed a dose related significant ($P < 0.01$) reduction in triglycerides compared to pretreatment levels (Table 2). MESC at the doses of 200 and 400 mg/kg was dose dependently reduced the Total cholesterol, LDL, VLDL, TG levels than diabetic control rats.

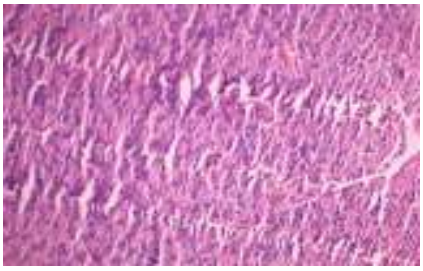
HISTOPATHOLOGICAL STUDIES



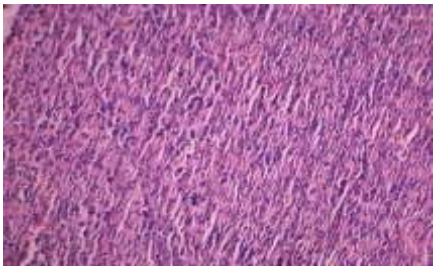
Group-I (Normal Control)



Group-II (Diabetic Control)



Group-III (MESC-200mg/kg)



Group-IV (MESC-400mg/kg)

Group-V (Glibenclamide - 600 µg/kg)

Table 1: Effect of *Smilax chinensis* L. on fasting blood glucose levels of STZ- induced diabetic rats

Groups (n=6)	Fasting Blood Glucose Levels			
	0^{th} Day	5^{th} Day	10^{th} Day	15^{th} Day
Group-I (Normal Control)	95 ± 1.15	95.66 ± 1.14**	95.16 ± 0.79**	95.5 ± 0.76**
Group-II (Diabetic Control)	279.5 ± 2.47	272.33 ± 2.19	282.33 ± .04	293.66 ± 2.96
Group-III (MESC-200mg/kg)	276.5 ± 2.75[a]	231.5 ± 1.97**[a]	167.66 ± 2.46**[a]	123.16 ± .41**[a]
Group-IV (MESC-400mg/kg)	282.16 ± 3.89[a]	229 ± 3.44**[a]	161.83±1.04**[a]	120.5 ± 1.28**[a]
Group-V (Glibenclamide - 600 µg/kg)	281.5 ± 3.05[b]	206.33 ± 2.24**[b]	138.16 ± 3.56**[b]	100 ± 0.66**[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 and ns-non significant. 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 2: Effect of *Smilax chinensis L.* on Total lipid profile of STZ- induced diabetic rats

Groups (n=6)	Biochemical Parameters				
	TG (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group-I (Normal Control)	80.72±0.43**[a]	90.86±0.17**[a]	34.39±0.50**[a]	33.14±0.63**[a]	15.01±0.90**[a]
Group-II (Diabetic Control)	137.57±1.10	152.72±5.89	15.87±4.19	75.52±4.19	48.2±5.12
Group-III (MESC-200mg/kg)	112.45±0.59*[b]	97.58±0.44**[b]	25.39±0.25**[b]	38.65±0.59**[b]	28.52±0.44**[b]
Group-VI (MESC-400 mg/kg)	95.00±0.47**[b]	89.04±0.49**[b]	27.93±0.39**[b]	35.07±0.35**[b]	24.07±0.47**[b]
Group-V (Glibenclamide - 600 µg/kg)	84.14±0.61**[c]	82.00±0.49**[c]	28.71±0.34**[c]	32.17±0.25**[c]	20.52±0.58**[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 and ns-non significant. 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.

Discussion and conclusion

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs (Grover *et al*., 2000). The aim of the present study was to evaluate the anti-diabetic effects of *Smilax chinensis L.* in STZ-induced diabetic rats. The experimental diabetic model used in this study was type 2 since low dose of STZ (55 mg/kg bw) destroyed some population of pancreatic beta cells (Aybar *et al*., 2002). There were residual beta cells which secreted insufficient insulin causing type 2 diabetic model (Gomes *et al*., 2001).

The mechanism by which streptozotocin STZ, a highly cytotoxic agent of pancreatic β-cells (Elsner *et al*., 2000), induces diabetes by damaging the cells that causes reduction in insulin. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of *Smilax chinensis L.* The reduced glucose levels suggested that *Smilax chinensis L.* might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis (Ali *et al.,* 1993; Gray *et al.,* 2000), or by absorption of glucose into the muscle and adipose tissues (Kamanyi *et al*., 1994) through the stimulation of a regeneration process and revitalisation of the remaining beta cells (Shanmugasundaram *et al*., 1990; Rokeya *et al.,* 1999; Bolkent *et al*., 2000).

The possible mechanism by which MESC mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. In this context, a number of other plants have been reported to have antihyperglycemic activity with a stimulatory effect on insulin release (Esmaeili and Yazdanparas, 2004; Sharma *et al.,* 2006). Since the extract produced highly significant antihyperglycemic effect even in streptozotocin-induced diabetic rats in which most of the β- cells are damaged, it is likely that MESC might have extra pancreatic mechanism of action. The other effects such as increase in the levels of total cholesterol, triglycerides and LDL-cholesterol and decrease in HDL-cholesterol of eremanthin in STZ-treated animals could be secondary to a partially restored beta-cell function with increased insulin levels. From the results of the present study, it may be suggested that the mechanism of action of MESC may be similar to glibenclamide action.

In diabetes, hyperglycemia is accompanied with dyslipidemia (Bierman *et al.,* 1966; Garber, 2002), i.e. characterized by increase in TC, LDL, VLDL, TG and fall in HDL. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes (Ananthan *et al.,* 2003). This altered serum lipid profile was reversed towards normal after treatment with the MESC. MESC exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in streptozotocin - induced diabetic rats. However, MESC

was found to be more effective in reducing the levels of TG and LDL as compared to its effect on TC. The elevated atherogenic index, i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk (Grover et al., 1999), was also shifted towards normal after MESC treatment. Thus, it is reasonable to conclude that MESC could modulate blood lipid abnormalities.

Summarizing, it could be proofed that the traditional use of *Smilax chinensis L.* as a hypoglycaemic agent is justified and that extracts from this plant show a dose-dependent activity which is comparable to the standard hypoglycaemic drug glibenclamide. Further studies to isolate, identify and characterize the active principle(s) are in the progress.

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