Journal of Pharmacopuncture

ISSN 2093-6966 [Print], **ISSN 2234-6856** [Online]
Journal of Pharmacopuncture 2013;16[3]:023-029
DOI: http://dx.doi.org/10.3831/KPI.2013.16.020

Original article

Antihyperlipidemic Activity of the Ethyl-acetate Fraction of *Stereospermum Suaveolens* in Streptozotocin-induced Diabetic Rats

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Key Words
antihyperlipidemic, atherogenic index, coronary risk index, *Stereospermum suaveolens*, ethyl acetate fraction, STZ-diabetic rats

Abstract

Objectives: Dyslipidemia in diabetes mellitus is a significant risk factor for the development of cardiovascular complications. The aim of this study was to evaluate the effect of the ethyl-acetate fraction of an ethanolic extract from *Streospermum suaveolens* on lipid metabolism in streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced by intraperitonial injection of STZ (50 mg/kg). Diabetic rats were treated with an ethyl-acetate fraction orally at doses of 200 and 400 mg/kg daily for 14 days. On the 15th day, serum lipid profiles, such as total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL), and high-density lipoprotein (HDL), were estimated in experimental rats. The atherogenic (AI) and the coronary risk (CRI) indices were also evaluated.

Results: The ethyl-acetate fraction at doses of 200 and 400 mg/kg significantly ($P < 0.001$) and dose-dependently reduced serum cholesterol, triglycerides and LDL, but increased HDL towards near normal levels as compared to diabetic control rats. The fraction also significantly ($P < 0.001$) lowered the atherogenic index (AI) and coronary risk index (CAI) in a dose-dependent manner.

Conclusion: The present study demonstrated that the ethyl-acetate fraction of *Stereospermum suaveolens* exhibits a potent antihyperlipidemic activity in hyperglycemic rats and suggests that the plant may have therapeutic value in treating the diabetic complication of hyperlipidemia.

Received: May 03, 2013 **Accepted:** Jun 24, 2013

1. Introduction

Dyslipidemia is a metabolic complication of diabetes mellitus characterized by low levels of high-density lipoprotein-cholesterol (HDL-C), and high levels of total cholesterol (TC), triglyceride (TG) and low-density lipoprotein-cholesterol (LDL-C). These lipoprotein abnormalities are held to be responsible for considerable cardiovascular-disease-related morbidity and mortality [1]. The risk for cardiovascular disease is increased approximately 2 to 4 fold in patients with di-

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abetes mellitus compared with non-diabetic controls [2]. Therefore, the detection of dyslipidemia and its treatment to reduce the cardiovascular risk and its consequences are required in diabetic patients.

In spite of the presence of known antidiabetic and antihyperlipidemic medicines in the pharmaceutical market, plant drugs and herbal formulation [3, 4] are used with success to treat the above conditions because they are frequently considered to be less toxic and freer from side effects than synthetic medications. Many herbs and plant products have been used empirically as antidiabetic and antihyperlipidemic remedies [5]; however, searching for new antidiabetic drugs with antihyperlipidemic properties from natural plants is currently very important.

Stereospermum suavelolens Roxb. is a large deciduous tree of the family Bignoniaceae that is found throughout the moist parts of India. Traditionally, a decoction of the root is used for the treatment of inflammation, pain and asthma [6, 7]. The flowers mixed with honey are given orally for the control of hiccups [8]. In southern India, the bark is used in folk medicine for the treatment of diabetes [6]. The root extract is known to possess anticancer activity due to the presence of lapachol [6, 9]. Previous phytochemical studies showed the presence of lapachol, dehydro-α-lapachone [10], sterekunthal B, and stereochenols A and B [11] in the bark, and stereolensin [12], scutellarein, 6-hydroxy luteolin [13], dinatin, and dinatin-7-glucuroniside [14] in the leaves. Previous studies in our laboratory showed that crude ethanol extracts of *Stereospermum suaveolens* had antihyperglycemic effects in streptozotocin-induced diabetic rats [15]. A literature survey revealed no experimental evidence for the antihyperlipidemic effect of the ethyl-acetate fraction of the plant. Therefore, the present work was undertaken to explore the hypolipidemic potential of the ethyl-acetate fraction of ethanolic extracts of *Stereospermum suaveolens* in Streptozotocin-induced diabetic rats.

Figure 1 *Stereospermum suaveolens* (Roxb.) DC aerial part.

Figure 2 *Stereospermum suaveolens* (Roxb.) DC leaves with flowers.

Figure 3 *Stereospermum suaveolens* (Roxb.) DC Bark.

2. Materials and methods

2.1. Chemicals

Streptozotocin (STZ) was purchased from SISCO Research Laboratory, India. Glibenclamide was obtained from Prudence Pharma Chem, Ankeshwara, Gujarat, India. The solvents and chemicals used were analytical grade.

2.2. Plant Material

The plant was identified and authenticated by the Tropical Botanical Garden and Research Institute, Palode, Tiruvananthapuram district, Kerala, India, and a voucher specimen (TBS-1) has been deposited in our laboratory for further reference. The bark of *Stereospermum suavelolens* (Roxb.) was collected from Palode forest, Tiruvananthapu-

ram district, Kerala, India. The bark of the plant was dried under shade and powdered with a mechanical grinder.

2.3. Preparation of the crude plant extract and fractions, and preliminary phytochemical analysis

The coarse powder bark of *Stereospermum suavelolens* (500 g) was packed in a soxhlet extraction apparatus and extracted with 1.5 l of 95% ethanol at a temperature of 40-50℃ for 72 h. The extract was filtered and then concentrated to dryness in a rotary evaporator under reduced pressure at temperature of 40℃. Then, the crude ethanol extract of *Stereospermum suavelolens* (EESS) (100 g) was dissolved in distilled water (500 ml) and fractionated with ethyl acetate. The yield of the ethyl-acetate fraction was 20.45% w/w. A weighed amount of the ethyl-acetate fraction was suspended in 5% dimethyl sulfoxide (DMSO) in normal saline prior to oral administration. The preliminary phytochemical screening was performed for the ethyl-acetate fraction of EESS with the standard procedures [16-18] and the nature of the phytoconstituents were identified.

2.4. Animals

Male Wistar albino rats (weighing 180-200 g) and male Swiss albino mice (20-25 g) were purchased from M/S-Ghosh Enterprises, Kolkata, India. The animals were randomly grouped (n = 6) and housed in polyacrylic cages ($38 \times 23 \times 10$ cm^3) and were maintained under standard laboratory conditions (25 ± 2℃) with a dark and light cycle (14/10 h). They were allowed free access to a standard dry pellet diet (Hindustan Lever, Kolkata, India) and had *ad*-libitum acess to water. Ethical clearance was obtained from Jadavpur University's Animals Ethical Committee for using the animals in the present study.

2.5. Acute toxicity study

An acute oral toxicity study was performed as per Organisation for Economic Co-operation and Development (OECD) 423 guidelines [19]. Male Swiss albino mice (20-25 g) were randomly distributed in six groups of three each. The animals were fasted overnight, and the ethyl-acetate fraction was administered orally at a dose of up to 2000 mg/kg. The animals were closely observed for the first 24 h for any toxic symptoms and for 72 h for any mortality.

2.6. Experimental Design

Rats were fasted overnight before the induction of diabetes with STZ. A freshly-prepared solution of STZ (50 mg/kg) in 0.1-M cold citrate buffer (pH 4.5) was injected intraperitoneally in a volume of 1 ml/kg [20], and the control rats were injected with citrate buffer alone. Hyperglycemia was confirmed by the elevated fasting glucose levels in blood determined at 48 h and then on day 6 after injection. Rats with moderate diabetes exhibiting fasting blood glucose levels in the range of 260-325 mg/100 ml were selected for the studies.

Overnight fasted rats were divided into five groups of six each [21]. The animals were treated orally once daily for 14 consecutive days as follows:

Group I, nondiabetic controls: 5% DMSO in normal saline (5 ml/kg),

Group II, STZ-diabetic controls: 5% DMSO in normal saline (5 ml/kg),

Group III, STZ-diabetic rats: ethyl-acetate fraction (200 mg/kg),

Group IV, STZ-diabetic rats: ethyl-acetate fraction (400 mg/kg),

Group V, STZ-diabetic standard: glibenclamide (0.5 mg/kg),

The fasting blood glucose level of each animal was determined on days 1, 4, 7, 10, and 15 after the initiation of treatment.

2.7. Serum biochemical parameters and statistical analyses

On the 15th day, blood was collected from the overnight-fasted rats by retro-orbital bleeding using a microcapillary technique. Serum was separated and used for the determination of lipid profiles, such as total cholesterol (TC), triglycerides (TGs), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (using Automated Span Diagnostic Reagents, Mumbai, India).

The atherogenic index (AAI) was calculated according to the method of [22], and the coronary risk index (CRI) was obtained by the method of [23]. The AI and the CRI were calculated from the total and the HDL cholesterol by using the formulaAtherogenic index (AI) = LDL cholesterol, HDL cholesterol

$$\text{Coronary risk index (CRI)} = \frac{\text{Total cholesterol}}{\text{HDL cholesterol}}$$

The experimental data were expressed as means ± SEMs. The data were analyzed using ANOVA and Dunnett's test. The results were considered statistically significance if $P < 0.05$.

3. Results

The qualitative phytochemical analysis of the ethyl-acetate fraction revealed the presence of flavonoids, tannins, alkaloids, saponins, and glycosides. No mortality and no toxic manifestations were observed up to a dose of 2000 mg/kg. Further dosing was not performed to estimate the

LD_{50} (lethal dose) value. According to the OECD guidelines for acute toxicity, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified; hence, the drug is found to be safe. Based on the acute toxicity studies, doses of 200 and 400 mg/kg for the ethyl-acetate fraction have been selected as therapeutic doses.

Repeated oral administrations with a dose of 200 or 400 mg/kg of the ethyl-acetate fractions of EESS to STZ-induced diabetic rats significantly ($P < 0.001$) reduced the elevated fasting blood glucose levels when compared to those of the diabetic control rats. The effect of the ethyl-acetate fraction is comparable to that of glibenclamide (Table 1).

The changes in the levels of serum lipids in non-diabetic controls, STZ-induced diabetic controls and fraction-treated rats are illustrated in Table 2. Compared to the non-diabetic control rats, the TC, TG, and LDL levels were increased significantly ($P < 0.001$), and the HDL levels were decreased significantly ($P < 0.001$) in the STZ-induced diabetic control rats. Compared to diabetic control rats, treatment of STZ-induced diabetic rats with ethyl-acetate fractions of EESS for 14 days resulted in marked decreases in the serum TC, TG, and LDL, and increases in the HDL levels, and these results were statistically significant ($P < 0.05$ and $P < 0.001$, respectively).

The atherogenic and the coronary risk indices in the diabetic control group were significantly ($P < 0.01$) increased, but the ethyl-acetate fraction of EESS (200 and 400 mg/kg) caused significant ($P < 0.01$ and $P < 0.001$) reductions in the AI and the CRI values to levels that were comparable to that of Glibenclamide (Table 2).

4. Discussion

Diabetes mellitus, the third leading cause of death in modern society, is associated with profound alteration in the serum lipid and lipoprotein profile, which plays a significant role in the development of premature atherosclerosis, coronary insufficiency and myocardial infarction [1, 24]. Lipid profile abnormalities in diabetes are mediated through derangements in a variety of regulatory processes,

Table 1 Effect of ethyl-acetate fraction of *Stereospermum suaveolens* on glucose level in STZ- induced diabetic rats

Groups	Serum Glucose Levels (mg/dl)				
	1 day	5th day	7th day	10th day	15th day
Normal (5% DMSO in 0.9% NaCl, 5 ml /kg)	85.64 ± 1.99	85.84 ± 2.18	87.84 ± 2.18	87.84 ± 2.18	86.60 ± 1.91
STZ-induced diabetic control (50 mg/kg)	290.65 ± 1.86 [a,*]	294.80 ± 2.35 [a,*]	294.84 ± 2.18 [a,*]	294.84 ± 2.18 [a,*]	293.40 ± 2.42 [a,*]
STZ+ethyl-acetate fraction (200 mg/kg)	264.81 ± 1.96 [b,†]	247.86 ± 1.86 [b,†]	211.84 ± 2.18 [b,†]	211.84 ± 2.18 [b,†]	135.42 ± 1.99 [b,†]
STZ+ethyl-acetate fraction (400 mg/kg)	283.28 ± 1.16 [b,†]	226.56 ± 1.30 [b,†]	173.48 ± 2.34 [b,†]	173.48 ± 2.34 [b,†]	124.12 ± 1.13 [b,†]
STZ+glibenclamide (0.5 mg/kg)	286.50 ± 6.35 [b,†]	173.50 ± 6.35 [b,†]	141.72 ± 6.26 [b,†]	141.72 ± 6.26 [b,†]	115 ± 6.11 [b,†]

Values are given as mean ± SEM, 6 rats in each group,

[a,*] $P < 0.001$ as compared to normal control group,

[b,†] $P < 0.001$, when compared with STZ-treated control group.

Table 2 Effect of ethyl-acetate fraction of *Stereospermum suaveolens* on serum lipid profiles in STZ-induced diabetic rats

Groups	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	AI	CRI
Normal (5% DMSO in 0.9% NaCl, 5 ml /kg)	139.20 ± 2.51	102.84 ± 2.31	120.22 ± 2.71	48.61± 1.36	2.47	2.86
STZ-induced diabetic control (50 mg/kg)	272.41 ± 2.82 [a,*]	215.60 ± 1.63 [a,*]	216.26 ± 1.77 [a,*]	26.28 ± 1.07 [a,*]	8.22	10.36
STZ+ethyl-acetate fraction (200 mg/kg)	179.80 ± 7.83 [b,†]	138.60 ± 4.90 [b,†]	138.60 ± 2.31 [b,†]	36.20 ± 1.16 [b,†]	3.82	4.96
TZ+ethyl-acetate fraction (400 mg/kg)	149.83 ± 2.67 [b,‡]	116.03 ± 3.74 [b,‡]	124.45 ± 1.77 [b,‡]	44.10 ± 1.30 [b,‡]	2.82	3.39
STZ+glibenclamide (0.5 mg/kg)	142.76 ± 2.40 [b,‡]	109.83 ± 1.37 [b,‡]	119.93 ± 2.47 [b,‡]	46.33 ± 1.24 [b,‡]	2.58	3.08

Values are mean ± SEM, 6 rats in each group,

[a,*] $P < 0.001$ as compared to normal control group,

[b,†] $P < 0.05$, when compared with STZ-treated control group, [b,‡] $P < 0.001$, when compared with STZ-treated control group.

especially insulin deficiency, thereby rendering diabetic patients more prone to hypercholesterolemia and hypertriglyceridemia [25]. We previously demonstrated the antihyperglycemic activitity of the crude ethanol extract of the *Stereospermum suaveolens* plant in STZ-induced diabetic rats [15]. In the present study, we investigated the antihyperlipidemic effect of the ethyl-acetate fraction of *Stereospermum suaveolens* in STZ-induced diabetic rats for 14 days.

In our study, we observed that daily administration of the ethyl-acetate fraction (200 and 400 mg/kg) of EESS for 14 days significantly reduced hyperglycemia in a dose-dependent manner in STZ-induced diabetic rats when compared to diabetic control rats. This finding suggests that the ethyl-acetate fraction of EESS has a potent antihyperglycemic activity in diabetic rats, which corresponds to a previous finding that the crude ethanol extract reduced blood glucose level in STZ-induced diabetic rats [15].

Diabetes mellitus is often linked with hyperlipidemia with increased risk of coronary heart disease [26]. Induction of diabetes in rats by administration of STZ led to the development of dyslipidemia. In the present study, an increase in serum total cholesterol, triglycerides and LDL levels were observed in STZ-induced diabetic control rats. The hyperlipidemia in the diabetic control rats revealed a significant alteration in lipid metabolism.

Insulin deficiency is associated with hypercholesterolemia and hypertriglyceridemia due to metabolic abnormalities [27]. In normal conditions, insulin increases the receptor-mediated removal of LDL-cholesterol, and decreased activity of insulin during diabetes causes hypercholestrolemia. The increased concentration of cholesterol could result in a relative molecular ordering of the residual phospholipids, resulting in a decrease in membrane fluidity [28, 29]. The abnormal high concentration of serum lipids in diabetes mellitus is mainly due to an increase in the mobilization of free fatty acids from the peripheral fat depots [28] by lipolysis. Increased release of free fatty acids increases the production of ketone bodies and triglycerides synthesis. In the present investigation, triglycerides were increased significantly in the diabetic control rats. Insulin deficiency depletes the activity level of lipoprotein lipase, thus leading to abnormal lipoprotein metabolism in diabetes and resulting in hypertriglyceridemia [30].

Lipoproteins have the major role in the occurrence of premature atherosclerosis in diabetic patients [31]. The coronary risk is well established by the elevated levels of total cholesterol and especially LDL cholesterol [32]. LDL transports cholesterol from the liver to other peripheral tissues. A crucial step in the pathogenesis of atherosclerosis is believed to be oxidative modification of LDL-C [33]. In the present study, the LDL level was increased in streptozotocin-induced diabetes mellitus. The increase in the serum LDL-C level may result from glycosylation of the lysyl residues of apoprotein B, which leads to a decrease in LDL metabolism due to a decrease in the affinity of LDL for its receptors [34]. However, treatment with ethyl-acetate fractions of EESS (200 and 400 mg/kg) significantly reduced the serum total cholesterol, the triglycerides, and the LDL in STZ-induced diabetic rats. This result implies that the ethyl-acetate fraction of EESS may prevent or be helpful in reducing the complications of the lipid profile, as well as improving lipid metabolism in diabetes. This could be correlated with our previous study in which the crude ethanol extract of *Stereospermum suaveolens* was reported to have antihyperglycemic and antihyperlipidemic effects on diabetic animals [15].

HDLs protect against or reverse atherosclerosis by their ability to serve as acceptor particles for macrophage cholesterol efflux, prevention of endothelial dysfunction, and maintenance of endothelial integrity [35, 36]. A decrease in the HDL was observed in the present study on diabetic rats, which will increase the chances of atherosclerosis. Treatment with the ethyl-acetate fraction of *Stereospermum suaveolens* (200 and 400 mg/kg/day, p.o.) showed marked elevation in the HDL level as compared to that in the controls. The increase in HDL cholesterol is associated with a decrease in coronary risk [37]. The HDL level inversely correlates with the risk of atherosclerotic cardiovascular disease. The AI and the CRI were very high in the streptozotocin-induced diabetic control rats. The increase in the HDL-C level achieved by using the ethyl-acetate fraction significantly decreased the treated rats, atherogenic and coronary risk indices. Thus, the ethyl-acetate fraction has the potential to prevent the formation of atherosclerosis and coronary heart disease, which are secondary diabetic complications of severe diabetes mellitus. The result of this present study clearly shows that the ethyl acetate-fraction of *Stereospermum suaveolens* has lipid-lowering effects on serum triglycerides, total cholesterol and low-density lipoprotein cholesterol in STZ-induced diabetic rats. *Stereospermum suaveolens* treatment also increases the serum level of high-density lipoprotein cholesterol. The mechanism(s) of the antihyperlipidemic actions of the ethyl-acetate fraction of *Stereospermum suaveolens* are not known; however, they could be mediated by control of tissue metabolism, as well as improved insulin secretion and action, because insulin lowers lipid levels and normalizes lipids in STZ-induced diabetic rats.

However, previous studies have reported antihyperglycemic and antihyperlipidemic effects of flavonoids, tannins, alkaloids, saponins, and glycosides [38]. The presence of these phytoconstituents in the ethyl-acetate fraction in high concentrations could account for these observed

antihyperglycemic and antihyperlipidemic effects. Again, this hypothesis would require experimental validation.

Another observation drawn from this study is the relative oral safety of the extract at a dose of 2000 mg/kg. According to the OECD guidelines for the acute toxicity of any drug, an LD_{50} dose of 2000 mg/kg and above is categorized as unclassified; hence, the drug is considered to be of low toxicity and to be safe. Arising from this documented fact, a fraction at an oral dose of 2000 mg/kg could be considered relatively safe for acute oral exposure.

5. Conclusion

The present investigation clearly indicates that the ethyl-acetate fraction of an ethanol extract of *Stereospermum suaveolens* exhibits antihyperlipidemic, in addition to antihyperglycemic, effects in STZ-induced diabetic rats. Further research is going on to isolate, identify and characterize the active principle(s) and as to pinpoint the exact molecular mechanism of the ethyl-acetate fraction of EESS involved in antihyperlipidemic activity.

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