Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading

Antihyperlipidemic activity of alcoholic leaf extract of *Solanum surattense* in streptozotocin-diabetic rats

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ARTICLE INFO

Article history:
Received 15 April 2011
Received in revised form 27 April 2011
Accepted 28 June 2011
Available online 28 June 2011

Keywords:
Solanum surattense
Antihyperlipidemic
Diabetes
Fatty acid composition
Streptozotocin
Lipid profile
Biochemical estimation
Plasma lipoprotein
Anti-diabetic
Blood glucose
Plasma insulin
Hypolipidemic

ABSTRACT

Objective: To study the antihyperlipidemic efficacy of alcoholic leaf extract of *Solanum surattense* (*S. surattense*) in streptozotocin (STZ) induced diabetic rats. **Methods:** The male albino Wistar rats were randomly divided into five groups with six animals in each group. Diabetes was induced by intraperitoneal injection of STZ (40 mg/kg). After being confirmed the diabetic rats were treated with *S. surattense* leaf extract (100 mg/kg b.w.) for 45 days. The biochemical estimation like lipid profile and fatty acid composition of tissues was performed. **Results:** The diabetic rats showed elevated levels of blood glucose, and a significant decrease in plasma insulin. It also showed significant increase in the levels of total cholesterol (TC), triglyceride (TG), phospholipids (PL) and free fattyacids (FFA) in the plasma, liver and kidney. The plasma lipoproteins were changed in diabetic rats. High density lipoprotein cholesterol (HDL-C) decreased, low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) increased. Fatty acid compositions were also altered in STZ-diabetic rats. Palmitic, stearic and oleic acids increased and the levels of linolenic and arachidonic acids decreased. It also showed decreased levels of total proteins and albumin. Administration of *S. surattense* (100 mg/kg b.w.) to diabetic rats for 45 days significantly reversed the above parameters towards normalcy. **Conclusions:** The antihyperlipidemic effect is due to the presence of alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols in the extract. The hypolipidemic effect mediated by *S. surattense* may also be anticipated to have biological significance and provide a scientific rationale for the use of *S. surattense* as an anti-diabetic plant.

1. Introduction

Diabetes mellitus has a major impact on cardiac morbidity and mortality and cardiovascular diseases now account for 80% of all diabetic deaths[1]. Diabetes is known to affect large number of metabolic pathways, including lipid metabolism, by altering the activities of various enzymes involved in these pathways. Liver is an insulin dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes. The insulin deficiency causes excessive breakdown of lipid in adipose depots, resulting in increased level of free fatty acids (FAA)[2]. In addition, type 2 diabetic patients, often present with a concomitant atherogenic dyslipidemia *i.e.* elevated triglycerides (TG), low high density lipoprotein cholesterol (HDL-C), and elevated very low density lipoprotein cholesterol (VLDL-C) and low density lipoprotein cholesterol (LDL-C) that increases their risk of cardiovascular disease[3,4]. Earlier reports also show that fatty acids compositions of various tissues are altered in experimental and human diabetes[5,6].

Since, there is a high incidence of mortality for type 2 diabetics with their first myocardial infarction, aggressive therapy for treating diabetic dyslipidemia is recommended[3]. As currently available hypolipidemic agents lack desired properties of an ideal drug in combating the disease, there is an ongoing need for additional agents. Thus, researchers are involved to find out an effective,

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Foundation Project: Supported by Annamalai University, Chidambaram, Tamil Nadu with a university research fellowship [NO.H5/2006-2007 (dated 21.11.2006)].

safe, and less expensive drug from natural origin to treat dyslipidemia associated with hyperglycemia.

Many indigenous Indian medicinal plants have been found to be useful in successful management of diabetes. *Solanum surattense* (*S. surattense*) (family: Solanaceae, synonym: *Solanum xanthocarpum*) (Indian night shade) is commonly used in Indian traditional system for curing various ailments[7]. The fruit of this plant has been reported to possess antidiabetic potential in diabetic rats[8]. Recently we have reported the effect of *S. surattense* leaf extract on glycemic control, erythrocyte and tissue antioxidant status in streptozotocin (STZ)-diabetic rats[9,10]. In the present study, we analyzed the effect of *S. surattense* on lipids constituents and fatty acid composition in plasma and tissues of STZ-diabetic rats. The efficacy was compared with a standard hypoglycemic drug *i.e.* glibenclamide.

2. Materials and methods

2.1. Plant materials

Leaves of *S. surattense* were collected from local areas of Chidambaram Tamil Nadu, India. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India and a voucher specimen (AU 189) was deposited at the herbarium of botany.

2.2. Preparation of leaf extract

The plant leaf was shade dried at room temperature [(32±2) ℃] and the dried leaf was ground into fine powder using pulverizer. The powdered part was sieved and kept in deep freezer until the time of use. 100 g of dry fine powder was suspended in 400 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at (40±5) ℃.

2.3. Drugs and chemicals

Streptozotocin was obtained from Sigma-Aldrich Company (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck and HIMEDIA, Mumbai, India.

2.4. Animals

Male albino Wistar rats weighing (180-200 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University, and maintained in an air-conditioned room at (25±1) ℃ with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum*. All the experiments were conducted in Department of Biochemistry, Faculty of Science, Annamalai University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals[11] and the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No.160/1999/CPCSEA, Pro. No.360), Annamalai University, Annamalainagar.

2.5. Experimental induction of diabetes

The animals were made diabetic by an intraperitoneal injection of STZ (40 mg/kg b.w.) in a freshly prepared citrate buffer (0.1 M, pH 4.5) after an overnight fast. STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. The animals exhibited massive glycosuria which was determined by Benedict's qualitative test and hyperglycemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose concentration, 96 h after induction. Albino rats with a blood glucose level above 220 mg/dL were considered diabetic and were used in the experiment.

2.6. Experimental protocol

The animals were randomly divided into five groups with six animals in each group. *S. surattense* leaf extract was suspended in 2% gum acacia (vehicle solution) and fed by intragastric tube daily for 45 days.

The five groups were group I: normal control (2% gum acacia); group II: normal control + *S. surattense* (100 mg/kg b.w.) in 2% gum acacia; group III: diabetic control; group IV: diabetic rats + *S. surattense* (100 mg/kg b.w.) in 2% gum acacia and group V: diabetic rats + glibenclamide (600 μg/kg b.w.) in 2% gum acacia.

After 45 days of treatment, the 12 h-fasted animals were anesthetized between 8:00 a.m. and 9:00 a.m., using ketamine (24 mg/kg b.w.) (intramuscular injection) and sacrificed by decapitation. Blood was collected in tubes with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of blood glucose and in tubes with ethylenediaminetetra acetic acid (EDTA) for the estimation of total cholesterol (TC), TG, free fatty acids (FFA), phospholipids (PL), and HDL-C. Tissues such as liver and kidney were collected for the estimation of TC, TG, FFA, and PL.

2.7. Biochemical determinations

Blood glucose was estimated by the method of Trinder[12], using reagent kit. The insulin in the rat plasma was measured by the method of Burgi *et al*[13]. Plasma and tissue lipids were extracted by the method of Folch *et al*[14]. Plasma and tissue TC, TG, FFA, and PL were estimated by the methods of Siedel *et al*[15], Foster and Dunn[16], Falholt *et al*[17], and Zilversmit and Davis[18], respectively. Plasma HDL-C was estimated by the method of Warnick *et al*[19]. LDL-C and

VLDL-C were calculated by Friedwald's formula[20]. Plasma total protein and albumin were estimated by the methods of Gornall *et al*[21] and Corcoran and Durnan[22], respectively. Fatty acid methyl esters were extracted by the method of Morrison and Smith[23] and fatty acid composition was determined by gas liquid chromatography.

2.8. Statistical analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) by using statistical package of social science (SPSS) 10.0 for Windows. The significance level was set at $P<0.05$.

3. Results

Table 1 showed the level of plasma glucose and insulin of diabetic rats. Diabetic rats showed an increase in the blood glucose level and decrease in insulin level of diabetic rats. Administration of *S. surattense* and glibenclamide for 45 days significantly brought back these parameters towards normalcy.

LDL-C, VLDL-C, and HDL-C in the plasma of normal and STZ-diabetic rats were shown in Table 1. The diabetic rats had elevated levels of plasma LDL-C and VLDL-C, and decreased level of HDL-C. Administration of *S. surattense* and glibenclamide brought back serum lipid profiles towards normalcy.

Table 2 showed the levels of TC, TG, FFA, and PL in the plasma of normal and STZ-diabetic rats. A significant increase in the plasma lipids levels was observed in diabetic rats. Administration of *S. surattense* and glibenclamide brought back these parameters towards normalcy.

The levels of TC, TG, FFA, and PL in the liver and kidney of normal and STZ-diabetic rats were also shown in Table 2. Elevated levels of lipid contents were observed in diabetic rats. Administration of *S. surattense* and glibenclamide brought back these parameters towards normalcy.

Table 3 showed the alterations in the fatty acid composition of liver and kidney of normal and experimental rats. There was a significant elevation in palmitic acid (16:0), stearic acid (18:0) and oleic acid (18:1); whereas a significant decrease in linolenic acid (18:3) and arachidonic acid (20:4) was observed in tissues of diabetic rats. These altered fatty acid compositions were restored towards normalcy by *S. surattense* and glibenclamide treatment to diabetic rats.

Table 1
Effect of *S. surattense* leaf extract on blood glucose, insulin and lipid profile in plasma of normal and STZ-diabetic rats (means±SD).

Groups	Glucose (mg/dL)		Insulin (μU/mL)	Plasma (mg/dL)		
	Day 0	Day 45		HDL-C	LDL-C	VLDL-C
Group I	68.65±02.07	75.32±07.59^{a}	14.39±1.31^{a}	43.25±3.13^{a}	23.63±2.07^{a}	10.42±0.95^{a}
Group II	67.02±04.94	74.45±07.76^{a}	14.29±1.30^{a}	41.29±2.14^{a}	22.86±1.50^{a}	9.65±0.74^{a}
Group III	250.25±15.80	291.38±19.20^{b}	4.53±0.41^{b}	32.60±2.57^{b}	103.97±7.81^{b}	31.23±2.82^{b}
Group IV	247.95±17.92	140.57±12.82^{c}	8.97±0.80^{c}	37.03±2.19^{c}	36.16±3.25^{c}	23.08±1.26^{c}
Group V	254.09±14.60	105.22±8.68^{d}	13.88±1.26^{a}	41.38±2.29^{a}	29.16±2.54^{d}	11.79±1.06^{a}

Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT).

Table 2
Effect of *S. surattense* on TC, TG, FFA and PL in the plasma, liver and kidney of normal and STZ-diabetic rats (means±SD).

Groups	TC			TG			FFA			PL		
	Plasma (A)	Liver (B)	Kidney (B)	Plasma (A)	Liver (C)	Kidney (B)	Plasma (A)	Liver (C)	Kidney (C)	Plasma (A)	Liver (C)	Kidney (C)
Group I	77.31±3.53^{a}	3.54±0.24^{a}	4.12±0.30^{a}	52.11±4.79^{a}	4.28±0.38^{a}	4.00±0.42^{a}	54.88±4.54^{a}	6.80±0.51^{a}	3.26±0.30^{a}	78.20±4.52^{a}	20.22±1.61^{a}	15.21±1.24^{a}
Group II	73.80±3.47^{ac}	3.50±0.20^{a}	4.08±0.44^{a}	48.27±4.23^{a}	4.22±0.29^{a}	4.09±0.31^{a}	51.50±5.01^{ac}	6.26±0.49^{a}	3.67±0.32^{a}	80.01±7.84^{a}	21.44±1.65^{a}	14.10±1.35^{a}
Group III	167.81±10.24^{b}	5.70±0.35^{b}	8.28±0.66^{b}	156.19±14.14^{b}	6.43±0.58^{b}	7.22±1.02^{b}	138.48±10.46^{b}	15.20±0.66^{b}	9.66±0.61^{b}	159.99±11.62^{b}	58.66±4.21^{b}	31.77±1.24^{b}
Group IV	96.28±6.36^{d}	5.00±0.46^{c}	6.44±0.61^{c}	115.40±6.34^{c}	5.09±0.36^{c}	6.00±0.72^{c}	78.69±5.63^{d}	9.06±0.55^{c}	7.20±0.60^{c}	102.66±9.33^{c}	37.99±1.52^{c}	23.66±1.27^{c}
Group V	82.34±4.46^{ae}	4.19±0.18^{d}	5.35±0.64^{d}	58.94±5.37^{a}	4.35±0.45^{a}	4.71±0.49^{d}	60.79±5.06^{ae}	7.80±0.50^{d}	5.20±0.40^{d}	78.22±4.67^{a}	27.30±2.68^{d}	18.01±1.31^{d}

A: mg/dL; B: mg/g wet tissue; C: mg/100 g tissue. Values not sharing a common superscript differ significantly at $P<0.05$ (DMRT).

Table 3
Effect of *S. surattense* on fatty acid composition in the liver and kidney of normal and STZ-diabetic rats (means±SD) (%).

Groups	C16:0 Palmitic acid		C18:0 Stearic acid		C18:1 Oleic acid		C18:3 Linolenic acid		C20:4 Arachidonic acid	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Group I	22.10±2.0^{a}	23.80±2.17^{a}	11.33±1.03^{ad}	14.17±0.29^{ad}	8.84±0.80^{a}	6.12±0.55^{a}	6.80±0.62^{ad}	6.68±0.61^{a}	22.67±2.06^{a}	12.47±1.13^{a}
Group II	21.42±1.95^{a}	22.67±2.06^{a}	10.20±0.93^{a}	13.83±1.26^{a}	8.72±0.79^{a}	6.36±0.51^{a}	7.36±0.67^{a}	6.91±0.63^{a}	23.13±2.11^{a}	12.69±1.15^{a}
Group III	28.90±2.63^{b}	32.87±2.99^{b}	17.00±1.55^{b}	21.53±1.96^{b}	13.37±1.22^{b}	11.10±1.01^{b}	2.49±0.22^{b}	1.70±0.15^{b}	14.62±1.33^{b}	5.55±0.50^{b}
Group IV	26.07±2.37^{c}	28.79±2.62^{c}	15.30±1.39^{c}	18.13±1.65^{c}	10.42±0.95^{c}	9.06±0.82^{c}	5.44±0.49^{c}	3.96±0.36^{c}	17.00±1.55^{c}	7.36±0.67^{c}
Group V	23.23±2.11^{a}	24.94±2.27^{a}	12.47±1.13^{d}	15.87±1.44^{d}	9.18±0.83^{a}	6.80±0.62^{a}	6.23±0.56^{d}	5.66±0.51^{d}	20.40±1.86^{d}	10.20±0.93^{d}

Values not sharing a common superscript vertically differ significantly at $P<0.05$ (DMRT).

4. Discussion

The antihyperglycemic activity was associated with increase in plasma insulin. Though the exact mechanism of action of the extract is not known, it could be due to increased pancreatic secretion of insulin from existing β-cells[9]. The phytochemical screening of leaves shows the presence of a range of active pharmacological agents including alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols. Therefore, the phenolic and/or flavonoid compounds in the *S. surattense* extract may play a role in the control of hyperglycemia. These principles are known to be bioactive for the management of diabetes[24,25]. It is known that certain alkaloids and flavonoids exhibit hypoglycemic activity[26] and is also known for their ability of beta cell regeneration of pancreas[24,27]. Tannins have also shown to decrease blood sugar[28]. Thus, the significant antidiabetic effect of *S. surattense* may be due to the presence of more than one antihyperglycemic principle and/or their synergistic effects.

This is the first study to evaluate the antihyperlipidemic effect of *S. surattense* leaf extract in normal and STZ-induced diabetic rats. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia. In our study, administration of the *S. surattense* leaf extract to the STZ-diabetic rats significantly improved TC, TG and lipid profiles (LDL-C, VLDL-C and HDL-C) towards normalcy. The observed antihyperlipidemic effect may be due to decreased cholesterogenesis and fatty acid synthesis, and this may be also attributed to the enhanced glucose utilization. *S. surattense* enhances glucose metabolism by increasing glycolysis, and decreasing gluconeogenesis and glycogenolysis[9]. Various studies on medicinal plants have reported a similar lipid lowering activity[29]. The decreased level of FFA is also associated with decreased actions of lipolytic hormones, which, in turn, decreased the activity of hormone sensitive lipases on fat depots.

The elevated phospholipid levels in serum are a consequence of elevated lipoproteins. Good glycemic control and elevated levels of HDL-C and decreased levels of TG in the blood are significantly correlated with the phospholipid levels[30]. The serum cholesterol/phospholipids ratio is one of the important markers of development of atherosclerosis. The restoration of phospholipids by *S. surattense* leaf extract may be due to controlled mobilization of TG and improved insulin level secretion and action.

In the present study, the increased concentrations of palmitic acid, oleic acid and stearic acid in STZ-diabetic rats were well correlated with other reports[31]. The ability to provide sufficient amounts of long-chain polyunsaturated fatty acids (PUFA) such as arachidonic acid (20:4n-6) depends greatly on the conversion of linoleic acid (18:2n-6) to γ-linolenic acid (18:3n-6) *via* the rate-limiting enzyme Δ6-desaturase. Impaired in linoleic acid metabolism is associated with diabetic neuropathy and altered prostanoid synthesis[32-34] and has been implicated in the diabetes complications[35-38]. A significant decrease in the levels of PUFA may be attributed to the diminished activity of △6 desaturase and △9 desaturase activity (liver and kidney, respectively) in STZ-diabetic rat. This results in the impaired formation of linolenic acid and arachidonic acid. Thus the reduced availability of essential fatty acid intermediates in diabetes is further exacerbated by increased destruction due to elevated levels of reactive oxygen species[39]. The increased levels of free radicals induced by hyperglycemia lead to damage of plasma membrane, which results in degradation of phospholipids and polyunsaturated fatty acids. Shin *et al*[40] have reported that, insulin therapy restores fatty acid composition in STZ-diabetic rat tissues. Decreased free radical mediated oxidative stress by *S. surattense*[10] may also help in the restoration of linolenic acid and arachidonic acid in diabetic tissues, and insulin treatment may rapidly restore the △6-desaturase activity. The n-6 and n-3 PUFA are known to decrease thrombosis and are known to lower the incidence of cardiovascular diseases. Thus *S. surattense* and glibenclamide treatment brought back the levels of both saturated and unsaturated fatty acids towards normalcy in diabetic tissues, thus it may prevent diabetic vascular complications.

In conclusion, our results showed that *S. surattense* leaf extract markedly reduced dyslipidemia and hyperglycemia in STZ-induced diabetic rats. The hypolipidemic effect is due to the presence of phytochemicals such as saponins, flavonoids, phenolic compounds, glycosides and triterpenoids in the leaf extract which is in line with several authors[41].

The present investigation has also opened avenues for further research especially with reference to the development of potent phytomedicine for diabetes mellitus from *S. surattense* leaves. Further, studies are needed to purify the bioactive compounds in the extract, and use the purified compounds for bioassay-directed experiments.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The financial support to Dr. Sridevi M as university research fellowship from Annamalai University, Chidambaram, Tamil nadu, is gratefully acknowledged.

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