RESEARCH ARTICLE

ANTIHYPERLIPIDEMIC POTENTIAL OF A TRADITIONAL SIDDHA POLYHERBAL FORMULATION SUGNIL IN STREPTOZOTOCIN - INDUCED DIABETIC RATS

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ABSTRACT

We investigated antihyperlipidemic potential of a traditional siddha polyherbal preparation SUGNIL from a combination of nine Indian medicinal plants. The current study was undertaken to assess the therapeutic effect of this formulation on lipid profile changes in streptozotocin (STZ) induced diabetic rats with a view of finding out its possible effect on reducing vascular complications associated with diabetes mellitus. Oral administration of the polyherbal extract SUGNIL at a dose of 100mg/kg body weight per day to STZ induced diabetic rats for a period of 45 days resulted in a significant reduction in plasma and tissue (liver and Kidney) cholesterol, triglycerides, phospholipids and free fatty acids. In addition, the decreased plasma levels of high density lipoprotein–cholesterol and increased plasma levels of low density lipoprotein–and very low density lipoprotein–cholesterol in diabetic rats were restored to near normal levels following treatment with the polyherbal extract SUGNIL. Moreover, our preliminary quantitative drug analysis revealed the presence of vitamins (Vit C & Vit E) and phytochemicals (Flavonoids, tannins and phenols) in SUGNIL at various proportions. Therefore, it may be concluded that the presence of various medicinal plants and their active principles possessing single or diverse range of biological activities hold promise in developing polyherbal drug SUGNIL as a preventive measure in treatment of vascular complications associated with lipid abnormalities in diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a multi-factorial disease that has a significant impact on the health, quality of life expectancy of patients, as well as on the health care system. A survey on worldwide reported that presently there are more than 150 million diabetic patients and the number is expected to grow to more than 300 million people in the year 2025 [1]. Diabetes is characterized by hyperglycemia together with biochemical alterations of glucose and lipid metabolism [2]. These traits are hypothesized to be responsible for the changes in the circulatory and tissue lipids, that in turn play an important role in the development of micro- and macro-vascular complications [3]. To reduce the risk of late complications and negative outcomes of diabetes mellitus, such as coronary heart disease, peripheral vascular diseases and cerebro-vascular diseases, not only the control of blood glucose levels, but also lipid levels is necessary. To date, there are different groups of oral hypoglycemic agents for clinical use, having characteristic profiles of side effects [4].

Management of diabetes without any side effects is still a challenging one to the medical community. There is a continuous search for alternative drugs. Therefore it is prudent to look for options in herbal medicine for diabetes as well. India is in the cutting edge of well-recorded and traditionally well practiced knowledge of herbal medicine. More than 80% of India's 1.1 billion population still use traditional folk medicine therapies for treating their ailments [5]. In the traditional system of Indian medicine, plant formulation and combined extracts of plants are used as drug of choice rather than Individual. The herbal Ingredients in these formulations are selected based on their healing property with respect to the disease condition. There are several such medicinal plant formulations and recommendations for their preparation which are mentioned in antique literature for treating various ailments with no side effects to the individual. Since, the information is too old, there arises the need to revive these recommendations carefully by making use of modern scientific tools in order to advocate their scientific merit and supremacy over the existing drugs. WHO also recommended for further investigation into traditional method of treatment [6].

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With this background, a traditional siddha polyherbal formulation meant to treat diabetes have been revived from antique literature and manufactured as a drug in capsule form by the brand name SUGNIL and is being undertaken for current investigation. In our previous study, the same formulation has been screened for its antidiabetic potency in streptozotocin - induced diabetic rats. The experimental results were highly encouraging, because they revealed that the polyherbal formulation exhibit significant and consistent antihyperglycemic action with an additional advantage of reducing diabetic complications mediated through oxidative stress. In the present study, we investigated the effect of this formulation on lipid profile changes in normal and STZ induced diabetic rats with a view of finding out its possible effect on reducing vascular complications associated with diabetes mellitus.

MATERIALS AND METHODS

Preparation of polyherbal formulation SUGNIL

The polyherbal drug SUGNIL was prepared by siddha drug manufacturing unit "Naturo Herbal Remidies" Salem, Tamilnadu. The formula for its preparation has been revived from ancient literature. The medicinal plants used in drug preparation were purchased from local market (Shanmugam and Sons, Salem) and authenticated (Table 1). All plants were shade dried, powdered and different proportions were mixed thoroughly. The mixture was further boiled in distilled water (3Litres) at 100°C for 60 minutes and filtered. The filtrate was evaporated to dryness and lyophilized. A known amount of solvent free extract was freshly suspended in sterilized water each time and administered intragastrically. The dosing schedule used was once per day.

Animals used

Male albino rats of the Wistar strain, weighing approximately 180-200 g, were used in the present study. Rats were acclimatized to the laboratory conditions for at least a week before any experimental work was undertaken. Rats were fed *ad libitum* with a normal laboratory pellet diet and water. The experiments were designed and conducted according to ethical norms approved by the Institutional Animal Ethics Committee of Animal care, Raja Muthiah Medical College, Annamalai Nagar.

Induction of experimental diabetes

Rats were fasted for 16 h prior to induction of diabetes by intraperitoneal injection of 55mg/kg body weight STZ (Sigma, St Louis, MO, USA) freshly dissolved in 0.1mol/L cold sodium citrate buffer, pH 4.5 [7]. Control rats received equivalent amounts of buffer intraperitoneally. Animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Hyperglycemia was confirmed 1 week after induction via blood glucose level measurements after a 16 hours fast. Animals with a fasting blood glucose level greater than 250 mg/dL were considered diabetic and included in the present study.

Experimental Procedure

Rats were divided into four groups, with six rats in each group, as follows: (i) group-I, control rats;(ii) group-II, STZ–

induced diabetic control rats; (iii) group-III, diabetic rats given polyherbal formulation SUGNIL (100 mg/kg body weight) in aqueous solution daily via intragastric tube for 45 days; (iv) group-IV, control rats given polyherbal formulation SUGNIL (100 mg/kg body weight) in aqueous solution daily via intragastric tube for 45 days.

After 45 days, 16 hours fasted rats were killed by cervical dislocation under mild anesthesia. Blood was collected on decapitation and serum was separated by centrifugation at 2500 rpm for 15 minutes. The liver and kidney were dissected out, washed immediately in ice cold saline and homogenized in Tris Hcl buffer, pH 7.4 (0.1 mol/L) with a Teflon homogenizer. Total lipids were extracted from the tissue homogenate according to the method of Folch *et al*. [8].

Analytical Methods

The cholesterol content in plasma, liver and kidney was estimated according to the method of Zlatkis et.al.(1953) [9], triglycerides were estimated according to the method of Foster and Dunn (1973) [10] and free fatty acids were determined according to the method of Falholt et.al (1973) [11]. Total phospholipid content was estimated according to the method of Bartlette [12] after digestion with perchloric acid and the phosphorus liberated was estimated as described by Fiske and Subbarow [13]. High-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were separated from the plasma using the dual-precipitation technique [14] and the cholesterol content of the lipoproteins was estimated.

Statistical analysis

All grouped data were evaluated statistically with spss/7.5 softwere (SPSS, Chicago, IL, USA). Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. $P<0.05$ was considered significant. All results are expressed as the mean $\pm$ SD for six animals in each group.

RESULTS

Table 2 depicts the levels of serum total cholesterol, triglycerides, phospholipids and free fatty acids in control and experimental group of rats. A significant ($P<0.05$) increase in the levels of plasma lipids was observed in diabetic rats (Group II) which were restored, to near normal by the treatment of polyherbal formulation SUGNIL (Group III). However, there was no significant effect witnessed on control rats treated with SUGNIL (Group IV). Fig.1 shows the levels of serum HDL, LDL and VLDL-Cholesterol in control and experimental groups of rats. The levels of LDL and VLDL-Cholesterol were significantly ($P<0.05$) increased, whereas the HDL-Cholesterol was markedly ($P<0.05$) decreased in rats induced with STZ (Group II) when compared with control rats (Group I). The diabetic rats treated with SUGNIL (Group III) for 45 days, LDL and VLDL-Cholesterol were significantly reduced, where as the HDL-Cholesterol was significantly increased. The control treated rats have no significant difference (Group IV). Comparison of liver lipid contents in control and experimental groups of rats are shown in Table 3. A significant increase ($P<0.05$) in the levels of total

Table 1. SUGNIL formula (composition and concentration)

S.No	Botanical name	Family	Part used	Concentration (mg/dl)
1.	*Aristolochia bracteata*	Aristolochiaceae	Whole plant	40
2	*Shorea roxburghii*	Dipterocarpaceae	Gum	40
3.	*Cassia auriculata*	Ceasalpinaceae	Flower	50
4.	*Casearia esculanta*	Asclepiadaceae	Leaf	40
5.	*Coscinium fenestratum*	Menispermaceae	Bark	50
6.	*Curcuma longa*	Zingiberaceae	Tubers	40
7.	*Eugenia jambolana*	Myrtaceae	Seeds	50
8.	*Gymnema sylvestre*	Asclepiadaceae	Leaves	50
9.	*Triphala*	Euphorbiaceae	Fruits	40

Table 2. Levels of serum total cholesterol, triglycerides, phospholipids and free fatty acids in control and experimental groups of rats

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	Phospholipids (mg/dl)	Free fatty acids (mg/dl)
I-Control	80.93 ± 3.35	67.24 ± 4.68	81.66 ± 2.07	55.17 ± 3.48
II-Diabetic control	$175.07 \pm 3.54^*$	$132.8 \pm 7.48^*$	$168.53 \pm 14.14^*$	$146.64 \pm 4.70^*$
III-Diabetic+SUGNIL (100 mg/kg)	$90.27 \pm 5.43^\dagger$	$79.31 \pm 6.1^\dagger$	$114.38 \pm 9.6^\dagger$	$65.16 \pm 4.86^\dagger$
IV-Control+SUGNIL (100 mg/kg)	7.93 ± 2.73	61.05 ± 2.54	79.94 ± 3.07	51.56 ± 4.52

Data are the mean ± SD for six animals in each group. *p<0.05 compared with control rats, †p<0.05 compared with diabetic control rats.

Table 3. Levels of liver total cholesterol, triglycerides, phospholipids and free fatty acids in control and experimental groups of rats.
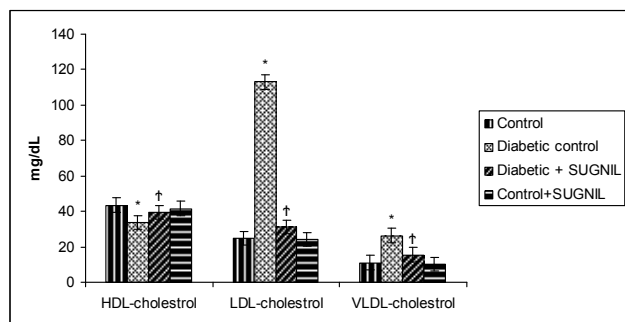
Groups	Total cholesterol (mg/g)	Triglycerides (mg/g)	Phospholipids (mg/g)	Free fatty acids (mg/g)
I-Control	4.35 ± 0.36	5.81 ± 0.44	22.70 ± 1.63	6.13 ± 0.10
II-Diabetic control	$6.58 \pm 0.30^*$	$7.35 \pm 0.34^*$	$62.16 \pm 3.77^*$	$13.07 \pm 0.15^*$
III-Diabetic+SUGNIL (100 mg/kg)	$4.93 \pm 0.13^\dagger$	$6.18 \pm 0.10^\dagger$	$32.43 \pm 3.02^\dagger$	$7.00 \pm 0.16^\dagger$
IV-Control+SUGNIL (100 mg/kg)	4.33 ± 0.39	5.89 ± 0.23	22.14 ± 1.56	6.10 ± 0.19

Data are the mean ± SD for six animals in each group. *p<0.05 compared with control rats, †p<0.05 compared with diabetic control rats.

Table 4. Levels of kidney total cholesterol, triglycerides, phospholipids and free fatty acids in control and experimental groups of rats

Groups	Total cholesterol (mg/g)	Triglycerides (mg/g)	Phospholipids (mg/g)	Free fatty acids (mg/g)
I-Control	5.19 ± 0.16	5.46 ± 0.38	15.62 ± 1.14	4.12 ± 0.30
II-Diabetic control	$9.17 \pm 0.25^*$	$8.26 \pm 0.32^*$	$39.10 \pm 1.84^*$	$8.14 \pm 0.32^*$
III-Diabetic+SUGNIL (100 mg/kg)	$5.83 \pm 0.18^\dagger$	$6.11 \pm 0.23^\dagger$	$17.84 \pm 1.01^\dagger$	$4.02 \pm 0.33^\dagger$
IV-Control+SUGNIL (100 mg/kg)	5.05 ± 0.11	5.16 ± 0.21	15.15 ± 1.18	6.13 ± 0.30

Data are the mean ± SD for six animals in each group. *p<0.05 compared with control rats, †p<0.05 compared with diabetic control rats.

Data are the mean ± SD for six animals in each group. *p<0.05 compared with control rats, †p<0.05 compared with diabetic control rats

Fig 1. Levels of HDL ,LDL and VLDL-cholesterol in control and experimental groups of rats

cholesterol, triglycerides, phospholipids and free fatty acids were observed in diabetic rats (Group II) when compared with control rats (Group I). Administration of SUGNIL to diabetic rats tends to bring the levels of hepatic lipids to near normal level. The control treated rats have no significant difference (Group IV). The levels of total cholesterol, triglycerides, phospholipids and free fatty acids in kidney of control and experimental groups of rats are shown in Table 4. A Significant increase (P<0.05) in the kidney lipid contents was observed in diabetic rats (Group II) when compared to control rats (Group I). Administration of SUGNIL of diabetic rats

tends to bring the levels of kidney lipid contents to near normal level. However, the control rats showed no effect upon SUGNIL treatment (Group IV).

DISCUSSION

Streptozotocin (STZ), a β-Cytotoxin, induces 'chemical diabetes' in a wide variety of animal species including rats by selectively damaging the insulin secreting β cells of pancreas, which leads to a reduction of insulin release [15]. It is well exposed from our pervious study results that the antihyperglycemic activity of SUGNIL was associated with an increase in plasma Insulin, suggesting that the drug stimulates insulin secretion from the remnant β cells and/or from regenerated β cells, and there by expresses insulinogenic effect. In STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, triglycerides, LDL and VLDL and decreases in HDL [16]. Activation of hormone-sensitive lipase (HSL) during insulin deficiency is accompanied by enhanced release of free fatty acids from adipose tissue [17]. Thus, excess fatty acid in the plasma produced by the STZ-induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess triglycerides formed in the liver, may be discharged into the blood in the form of lipoproteins

[18]. The observed increase in plasma phospholipids is a consequence of elevated lipoproteins. Therefore, the marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots. However treatment with the polyherbal extract SUGNIL normalized plasma lipid status, which was presumably mediated by a control of lipid metabolism.

The liver is an important insulin-dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes [19]. Liver tissue participates in the uptake, oxidation and metabolic conversion of fatty acids, the synthesis of cholesterol and phospholipids and the secretion of specific classes of serum lipoproteins. In diabetes, fatty acids are increasingly taken up by the liver and, after esterification with glycerol phosphate, they are deposited as triglycerides. As a result, diabetic liver steatosis develops [20]. Furthermore, the accumulation of triglycerides and long-chain fatty acyl coenzyme A (CoA) in the liver leads to reduction in insulin mediated metabolic activity and can cause type 2 diabetes, resulting in metabolic syndrome [21]. 3-Hydroxy-3-methylglutaryl CoA reductase catalyses the rate-limiting step in cholesterol biosynthesis and its activity was found to be significantly increased in the liver of diabetic rats [22]. The increase in liver cholesterol in diabetic rats observed in the present study could be due to increased cholesterogenesis. The present study showed a decrease in liver cholesterol, triglycerides, phospholipids and free fatty acids in diabetic rats after treatment with the polyherbal extract SUGNIL. This reduction may be attributed to increased clearance and decreased production of the major transporters of endogenously synthesized cholesterol and triglycerides.

Diabetes mellitus affects the kidney and is the leading cause of diabetic nephropathy. Abnormal lipid metabolism and the renal accumulation of lipids have also been proposed to play a role in the pathogenesis of diabetic nephropathy [23]. Several studies have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and have proposed that these deposits may play an important role in the pathogenesis of diabetic kidney disease [24]. The elevated levels of renal lipid contents observed in diabetic rats could be due to increased renal lipid synthesis. In the present study, the polyherbal extract SUGNIL was able to significantly decrease the concentration of these lipids in treated diabetic rats compared with untreated diabetic rats. This reduction could be beneficial in preventing diabetic complications, as well as in improving lipid metabolism in diabetic kidney [25].

Fatty acids, an important component of cell membranes, are eicosonoid precursors and are therefore required for both the structure and functions of every cell in the body. Many studies have focused on disorders of lipid metabolism, especially alterations in tissue fatty acids in diabetes. In the present study, we observed significant alterations in the fatty acid composition in tissues of diabetic rats. Normalization of tissue fatty acids following the administration of polyherbal extract SUGNIL may be attributed to the decrease in plasma lipids caused by the extract, which results in decreased synthesis of fatty acids. Furthermore, normoglycaemia and inhibition of lipolysis may lead to decrease in the synthesis of fatty acids in diabetic tissues.

Overall, the results indicate that the presence of various medicinal plants and their diverse pharmocological actions work together in holistic way and increases the therapeutic efficacy of SUGNIL. This is in agreement with the fact that, the active medicinal plant ingredients of SUGNIL viz. *Gymnema sylvestre* [26], *Eugenia jambolana* [27], *Cocinium fenestratum*[28], *Shorea roxburghii* [29], *Curcuma longa* [30], *Cassia auriculata* [31] and *Triphala* [32], have already been reported to have antihyperlipidemic effect in different animal models. Moreover, from our previous study it has been reported that the preliminary quantitative drug analysis revealed the presence of vitamins like C & E and phytochemicals such as flavonoids, tannins and phenols, in SUGNIL. Vitamins C & E, both are reported to have cardioprotective effect [33,34] and tannins, flavonoids and phenols have hypolipidemic and hypocholestreolemic effects [35,36,37]. Thus, the antioxidants present in the SUGNIL may be responsible, in part, for the antihyperlipidemic effect of the SUGNIL. Therefore, it may be concluded that the active principles possessing single or diverse range of biological activities hold promise in developing polyherbal drug SUGNIL as a preventive measure in treatment of vascular complications associated with lipid abnormalities in diabetes mellitus.

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