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SCREENING OF ANTI-DIABETIC POTENTIAL OF THE SIDDHA FORMULATION SARABENDIRA SIDDHA MARUTHUVA SUDAR CHOORANAM STREPTOZOTOCIN INDUCED TYPE II DIABETES IN WISTAR RATS

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ABSTRACT

R Diabetes mellitus is a chronic metabolic disease which may be suspected or recognized clinically by the onset of one or more of the characteristic symptoms such as polyuria, polydipsia, polyphagia and unsolved weight loss. Studies have shown large regional and socioeconomic differences in the prevalence of type II diabetes (T2D) in India. A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is 350 million. Effective control of plasma glucose levels is an effective strategy to prevent the diabetic complications and improving quality of life in diabetic patients. Nowadays, the management of T2D has become increasingly complex and, to some extent, controversial, with the wide range of available anti-diabetes medication. Both sulfonylureas and glinides are the first line gold standard treatment available for management of type II diabetes. But loss of efficacy, hypoglycemia, hypersensitivity, dizziness, GI disturbance, and weight gain represent the main problems related to the use of these drugs. The global use of complementary and alternative medicine for the management of diseases such as diabetes has rapidly increased over the last decade. It is reported that up to 72.8% of people with diabetes used herbal medicine, dietary supplements and other alternate therapies. Siddha system of medicine also join hands in treating diabetic cases since centuries back. The main aim of the present study is to evaluate the anti-diabetic potential of siddha formulation sarabendira siddha maruthuva sudar chooranam (SBSMSC) in streptozotocin (STZ) induced diabetic rats. Treatment with SBSMSC at both the dose level of 500 and 1000 mg/kg has remarkable reduction on blood glucose, improved insulin production with significant control on HbA1C, serum urea and creatinine level, these results were well justified with the histopathological findings. It was concluded from the results of the present study that the siddha formulation SBSMSC reveals significant anti-diabetic property on the tested animals. Being an polyherbal preparation it may be well tolerated upon clinical usage for the management of T2DM in patients.

KEY WORDS: ***Siddha, Sarabendira siddha maruthuva sudar chooranam, Anti-diabetic potential, Streptozotocin, Type II diabetes, HbA1C, Metabolic disease***

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1. Introduction

Diabetes mellitus (DM) has reached epidemic proportions globally [1]. The World Health Organization (WHO) estimated that there were 135 million diabetic individuals in the year 1995 and it has been projected that this number will increase to 300 million by the year 2025 [2]. WHO has projected that the maximum increase in the number of diabetics would occur in India. Considering the large population and increasing prevalence of diabetes mellitus of nearly 33 million diabetic subjects, the burden of diabetes in India could be enormous [3].

Evidence suggests that lifestyle changes such as exercise, diet and other nonpharmacological interventions can delay and even prevent the development of T2DM. However, compliance to these interventions is low; with only about 50% of those with chronic illnesses have been shown to adhere to recommended lifestyle interventions [4]. Many antidiabetic pharmaceutical drugs are available, but the increase in the incidence of T2DM, especially in developing countries, together with adverse events associated with these drugs, has highlighted the need for more effective, safer and less costly management approaches.

There are three currently available agents, acarbose, miglitol and voglibose[5]. Their properties are different from other antidiabetics owing to its unique mode of action. Acarbose has been used for over 20 years in the treatment of hyperglycaemia [6]. side effects are mainly gastrointestinal and include flatulence, diarrhoea and abdominal pain. These symptoms are usually mild, but they may reduce compliance and they are the most common reason for discontinuation treatment [7]

The interplay of herbs and human health has been documented for thousands of years [8]. Herbs have been integral to both traditional and non-traditional forms of medicine dating back at least 5000 years [9,10]. The enduring popularity of herbal based medicines may be explained by the tendency of herbs with respect to its potency, biocompatibility, safety, economical and easy availability.

Siddha formulation sarabendira siddha maruthuva sudar chooranam majorly comprises of the following ingredients such as Naaval (Syzygium cumini),Vilamichu (Plectranthus zizanioides),Vetiver (Vetiveria zizanioides) ,Lavangam (Syzygium aromaticum) ,Akkaragaram (Anacyclus pyrethrum), Konrai (Cassia fistula),Aavarai (Cassia auriculata) ,Thetran (Strychnos potatorum), Sirukurinjan (Gymnema sylvestre) and Seenthil (Tinospora cordifolia). This formulation has been indicated for treating diabetes mellitus type II as per the literature. But still now it has not been exploring for the claimed activity, hence the main aim of the present study is to explore the anti-diabetic potential of this indigenous siddha preparation sarabendira siddha maruthuva sudar chooranam (SBSMSC) in streptozotocin (STZ) induced diabetic rats.

2.Materials and Methods

2.1. Experimental Animals

Healthy adult Wistar albino rats of either sex weighing between 200-220 g were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air by air handling unit. A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ¬+ 2o C and relative humidity 50–65%. They were provided with food and water ad libitum. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of science and technology, Chennai, Tamil Nadu, India. The IAEC approval number: SU/CLATR/IAEC/X/088/2018.

2.2. Measurement of Oral glucose tolerance test (OGTT) [11]

Overnight fasted normal rats were randomly divided into 3 groups of each 6 animals

Group I - Animals received 0.1 % CMC., Group II - Animals received 500mg/kg of SBSMSC, Group III- Animals received 1000mg/kg of SBSMSC. Initial blood glucose sugar level was determined from overnight fasted animals. All the animals were given glucose (2 g/kg) 30 min after dosing. Blood samples were collected on (0 hr), 60 and 120 min. Blood glucose concentration was estimated by the glucose oxidase strip.

2.3. Animal grouping

The animals were grouped into four groups of 6 animals each. Group I (Control group) -received normal saline, Group II – Diabetic control rats administered with 45 mg/kg,i.p of STZ, Animals

belongs to group III received 45 mg/kg,i.p of STZ and treated with 500mg/kg of SBSMSC. Animals belongs to group IV received 45 mg/kg,i.p of STZ and treated with 1000mg/kg of SBSMSC.

2.4. Induction of Diabetes with Streptozotocin [12]

Streptozotocin (STZ), at a dose of 45 mg/kg body weight was dissolved in citrate buffer, injected intraperitoneal to induce diabetes. The animals will be fasted for 16hrs before prior to STZ injection, and after the injection 5% sucrose will be supplemented for 24hrs in order to prevent the animals from fatal hypoglycemia. One week after STZ injection, blood glucose level was checked using glucometer. The animals with a blood glucose level of more than 300 mg/dl were considered diabetic and included in the study.

2.5. Body Weight Measurement and Glucose estimation

The fasting blood glucose was measured on 0th, 14th and 28th day by glucose estimation strip. Body weight of the animals was measured before start of the study and also at the end of the study.

2.6. Biochemical Estimation [13,14]

At the end of the study, before sacrifice, the animals were fasted for overnight with free access to water. Animals were sacrificed with excess anesthesia. Blood samples were collected from retro orbital puncture and stored in clot activator coated test tubes for serum biochemical analysis. Pancreas sample were harvested and carefully investigated for gross lesions.

2.7. Histopathological Analysis [15]

Sample obtained were immersed in 10% formalin for 24 h-48h for histopathological examination. After standard processing, the cut tissue was embedded in paraffin (Leica TP1020 tissue processor) and cut into 5 µm thick sections in a rotary microtome (Leica RM2255 - Fully Automated Rotary Microtome). The sections were stained with haematoxylin-eosin (Merck). Histological measurement and photographs were taken with Olympus CX31, Trinocular Biological Microscope (magnification 10x & 40 x).

2.8. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA (GRAPH PAD PRISM 5 computer program). Results are expressed as ±SEM. The data were statistically analyzed by ONE WAY ANOVA followed by Dunnett's multiple comparison test. Probability P values < 0.05 were considered as significant.

3.Results

3.1. Effect of SBSMSC on body weight of control and STZ induced experimental rats

Body weigh measurement is one of the core parameter in the present study it was observed that there was significant decrease in the body weight of rats challenged with STZ alone, whereas treatment with trial drug SBSMSC at both the dose level has shown marked increase in the body weight of the experimental rats. As shown in Table 1.

Table 1: Effect of SBSMSC on body weight of control and STZ induced experimental rats

Group	Before Treatment	After Treatment
	Body Weight in gms (0th day)	Body Weight in gms (28th Day)
Control	207.3 ± 3.7	230.5 ± 3.2
Diabetic control (STZ 45 mg/kg,i.p)	214.3 ± 3.70	179.7 ± 4.24**
STZ+500mg/kg of SBSMSC	209.7 ± 3.19	189.5 ± 1.23*
STZ+1000mg/kg of SBSMSC	203 ± 1.71	205.2 ± 1.85**

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.2. Effect of SBSMSC on Oral Glucose Tolerance Test

In OGTT analysis there was a profound increase in the level of blood glucose level on the 60th min of the glucose challenged rats whereas treatment with SBSMSC reduced the glucose level from the threshold peak level. As shown in Table 2.

Table 2: Effect of SBSMSC on Oral Glucose Tolerance Test

Group	Blood glucose level (mg/dl)		
	0 Min	60 min	120 min
Glucose 2 g/kg	72.67 ± 0.80	152.8 ± 2.13	131 ± 0.96
Glucose +500mg/kg of SBSMSC	75 ± 1.52	139.3 ± 1.28*	119 ± 1.36*
Glucose +1000mg/kg of SBSMSC	76.67 ± 1.68	129.7 ± 2.1*	105.5 ± 2.32*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.3.Effect of SBSMSC on fasting blood glucose and plasma insulin level of control and STZ induced experimental rats

From the results of the present study it was observed that there was a consistent increase in glucose level of rats challenged with STZ between 14th to 28th days of experimental periods. Treatment with SBSMSC at both the dose level of 500 and 1000 mg/kg has remarkable reduction on blood glucose between the 14th to 28th day interval time periods. As shown in Table 3.

Table 3: Effect of SBSMSC on fasting blood glucose and plasma insulin level

Group	Fasting Blood glucose level (mg/dl)			Insulin (ng/ml)
	0th day	14th day	28th Day	
Control	74.67 ± 2.06	77.17 ± 1.19	78 ± 2.58	0.8433 ± 0.03
Diabetic control (STZ 45 mg/kg,i.p)	74.67 ± 2.15	401.8 ± 3.92**	407.8 ± 3.22**	0.3283 ± 0.01*
STZ+500mg/kg of SBSMSC	76.67 ± 2.21	367.7 ± 8.34**	301 ± 2.91*	0.425 ± 0.013
STZ+1000mg/kg of SBSMSC	72 ± 1.52	312.5 ± 5.8**	279.8 ± 2.96*	0.49 ± 0.02*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001.

3.4. Effect of SBSMSC on HbA1C, serum urea and serum creatinine level of Control and STZ induced experimental rats

There was a significant increase in HbA1C, Serum Urea and Creatinine level in STZ alone treated rats whereas treatment with SBSMSC at both the dose level has shown significant reversal of the above mentioned serological parameter's. As shown in Table 4.

Table 4: Effect of SBSMSC on HbA1C, serum urea and serum creatinine level

Group	HbA1C (% Hb)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
Control	5.733 ± 0.18	22.83 ± 1.5	0.4167 ± 0.04
Diabetic control (STZ 45 mg/kg,i.p)	14.6 ± 0.23*	71.83 ± 2.35**	1.567 ± 0.09*
STZ+500mg/kg of SBSMSC	11.4 ± 0.2*	51.67 ± 1.20*	1.1 ± 0.08*
STZ+1000mg/kg of SBSMSC	9.417 ± 0.13*	44.67 ± 2.1*	0.9 ± 0.05*

Values represent mean ± SEM of 6 experimental animals. * P< 0.05; ** P< 0.01; *** P < 0.001

3.5. Effect of SBSMSC on Histopathology of Rat Pancreas (H&E) Staining under low and high power magnification

Histopathological analysis has revealed that both exocrine and endocrine portion of the pancreas appears normal with prominent histology of islet of Langerhans and acinar zone in control group rats. Increased fibrosis with high level invasion of inflammatory cells though connective septae and islets were observed in the sample belongs to group II STZ alone treated rats. Increase degeneration with loss in beta cell density. Marginal increase in cellular density with mild signs of pyknosis were observed in sample belongs to group III rats. Increased number of islets with distinct border surrounded by exocrine part of the pancreas were observed in sample belongs to group IV rats. Regular acini cellular zone and proper arrangement of islet of Langerhans were observed in sample belongs to group IV. As shown in Figure 1.

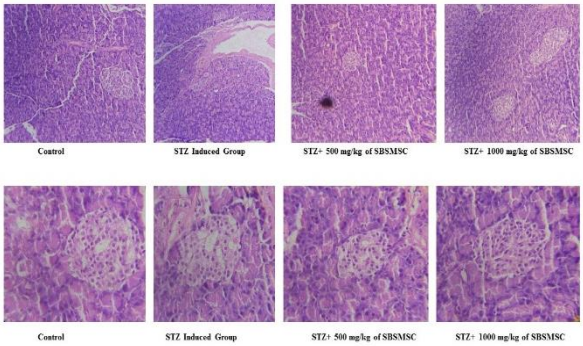

Figure 1: Histopathology of Rat Pancreas (H&E) Staining under low and high power magnification

4.Discussion

According to the International Diabetes Federation, in 2015, approximately 415 million people were suffering from diabetes worldwide, and this number is expected to exceed 640 million by the year 2040. It is estimated that half of patients with diabetes are unaware of their disease and are thus more prone to developing diabetic complications. However, the cost of dealing with diabetes can be unaffordable in terms of money spent and lives lost. In 2015, approximately 5.0 million deaths were attributed to diabetes, albeit in the same year, more than 12% of the global health expenditure was dedicated to coping with the disease and its complications [16].

Diabetes is induced by streptozotocin (STZ), a glucosamine–nitrosourea compound that is used clinically as a chemotherapeutic agent in the treatment of pancreatic β cell carcinoma. STZ damages pancreatic β cells, resulting in hypoinsulinemia and hyperglycemia [17]. STZ can induce a diabetic state in 2 ways, depending on the dose. The selectivity for β cells is associated with preferential accumulation of the chemical in β cells after entry through the GLUT2 glucose transporter receptor: chemical structural similarity with glucose allows STZ to bind to this receptor.

The glycemic index is an indicator of the postprandial blood glucose response to food per gram of carbohydrate compared with a reference food such as white bread or glucose. Hence, the glycemic load represents both the quality and quantity of the carbohydrates consumed [18].In the present study it was observed that there was a consistent increase in glucose level of rats challenged with STZ between 14th to 28th days of experimental periods. Treatment with SBSMSC at both the dose level of 500 and 1000 mg/kg has remarkable reduction on blood glucose between the 14th to 28th day interval time periods. Similar finding was observed in In OGTT analysis that there was a profound increase in the level of blood glucose level on the 60th min of the glucose challenged rats whereas treatment with SBSMSC reduced the glucose level from the threshold peak level

The majority of patients, however, belong to the group with insulin resistance as the core pathophysiological disorder rather than insulin deficiency, classified as type 2 DM. This type of diabetes is phenotypically often accompanied by central obesity, hypertension and dyslipidaemia [19]. In the present study it was observed that there was a significant decrease in insulin level in rats challenged with STZ. Treatment with SBSMSC at both the dose level of 500 and 1000 mg/kg has shown promising increase in plasma insulin level.

People with higher initial HbA1C levels had higher cumulative costs than people with lower levels, and people who experienced higher annual drift in HbA1C levels had even further increased costs [20].In the present study it was observed that there was a significant increase in HbA1C level in STZ alone treated rats whereas treatment with SBSMSC at both the dose level has shown significant reduction in the HbA1C level that provokes the excellent glycemic control by the drug.

Nephrotoxicity is a well-documented effects of STZ [21]. In addition to causing acidosis that can result from renal tubular damage in the kidney, STZ is one of the nitrosourea drugs or toxins known to cause type B lactic acidosis [22]. There was a significant increase in serum urea and creatinine level STZ alone treated rats whereas treatment with SBSMSC at both the dose level has shown significant reversal of the above mentioned serological parameter's.

The high concentration of blood glucose and other biochemical abnormalities result from a deficiency of β-cells of the endocrine pancreas and/or from a sub sensitivity to insulin in target cells [23]. Histopathological analysis has revealed that both exocrine and endocrine portion of the pancreas appears normal with prominent histology of islet of Langerhans and acinar zone in control group rats. Increased fibrosis with high level invasion of inflammatory cells though connective septae and islets were observed in the sample belongs to group II STZ alone treated rats. Increase degeneration with loss in beta cell density. Marginal increase in cellular density with mild signs of pyknosis were observed in sample belongs to group III rats. Increased number of islets with distinct border surrounded by exocrine part of the pancreas were observed in sample belongs to group IV rats. Regular acini cellular zone and proper arrangement of islet of Langerhans were observed in sample belongs to group IV.

5.Conclusion

Diabetes is a chronic metabolic disorders characterized by profuse hyperglycemia. Nearly 75% of subjects with diabetes mellitus live in low- and middle-income countries. In financial terms, the global burden of DM is enormous in the countries like India. Many traditional plants and herbal medicines have been found to possess the antidiabetic activity; however, the World Health Organization has recommended that traditional plant treatments for diabetes warrant further evaluation. Results of the present study clearly advocates the potential of the formulation SBSMSC with respect to the reduction of plasma glucose, improved insulin production along with significant control on HbA1C, serum urea and creatinine level in the treated rats. The reason behind the potency of the formulation might be due to presence of versatile phytotherapeutics and their bioactive components present in each herbal ingredients responsible for insulin secretagogue property. Hence it was concluded that the formulation like Sarabendira siddha maruthuva sudar chooranam may be considered as a primary drug of choice or as an additional supplementary therapy in treating patients with T2DM at clinical level. Further studies need to carried out to establish the exact molecular mechanism of drug action in treating diabetes.

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