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ANTI-DIABETIC POTENTIAL OF SIDDHA TRADITIONAL FORMULATION NAGA CHENDHOORAM IN STZ INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus (DM) is probably one of the oldest diseases known to man. Hallmark pathology of the all types of diabetes ends in hyperglycemia. In brief, it is now well known that profuse insulin deficiency accounts for about 10% of all DM cases and is characterized by selective autoimmune destruction of insulin producing pancreatic β-cells, which are classified as type 1 diabetes. Insulin sensitization is the issue prevails in type II. Patient is with diabetes are been prescribed with certain class of anti-hyperglycemic agents of which upon sustained usage the patients are subjected to side effects such as insomnia, diarrhea, drowsiness etc. Hence people are stared exploring the alternate medicine with preferably low or no side effects. Traditional therapy reveals appreciable remedy on managing the chronic metabolic disorders like diabetes. Traditional therapy possesses spectrum of drug categories like herbs, metals, minerals, poly herbals and herbomineral. Siddha system of medicine offers tremendous cure by adequately strengthen the physiology and regularize the metabolic mechanism, which becomes the key functional mechanism of majority of the siddha drugs. The main aim of the present study is to evaluate the anti-diabetic potential of siddha formulation Naga Chendhooram (NC) in streptozotocin (STZ) induced diabetic rats. Results of the study indicates that treatment with NC at both the dose level of 200 and 400 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. The use of traditional medicine offers greater therapeutic advantages, being imposed to current society in the urge to evaluate the mechanism of their underlying pharmacological action and their associated benefits and adverse effects. Thus, use of drugs like NC is still continued in modern society for the prevention, wellbeing and treatment of diabetes.

KEY WORDS: ***Diabetes mellitus, Type II, Traditional therapy, Siddha drug, Naga Chendhooram, Blood glucose, HbA1C.***

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1. Introduction

Diabetes Mellitus is a chronic disease which affects the metabolism of proteins, carbohydrates and lipids. The major characteristic is hyperglycaemia as a consequence of abnormal secretion of insulin in the pancreas (type I) or inefficient action of insulin in the target tissues (type II). Type 2 diabetes is sharply increasing globally, including in many parts of the developing world, in major part as a consequence of the worldwide "epidemic" of obesity. For centuries, prior to and after the discovery of insulin, medicinal plants have been used to normalize glycemia in diabetic patients. This disorder promotes adverse effects in all organic systems. Diabetes exerts a negative action on the neuroendocrine axis and those effects can enhance the action of diabetes on other organs that are dependent on the axis [1].

The dynamics of the diabetes and hyperlipidemia are changing rapidly in low- to middle-income countries. In 2030, diabetes may affect 472 million (approx.) of world populations. The number of adult with impaired glucose tolerance may rise from 344 million (2010) to 472 million by 2030 [2]. International diabetes federation (IDF) estimated that 80% of world diabetic population live in low- and middle-income countries in 2030. As per IDF 2011 report, China, India, and United States of America have 90.0, 61.3, and 23.7 million peoples were living with diabetes that may be increase up to 129.7, 101.2, and 29.3 million people, respectively, in 2030 [3].

Metformin is the drug of first-line for many patients with T2DM. It decreases fasting blood glucose by approximately 20% and HbA1c by 1.5%. It can be given in combination with sulfonylureas, glinides, alpha-glucosidase inhibitors, insulin, thiazolidinediones (TZD), glucagon-like peptide-1 receptor agonist (RA-GLP1), dipeptidylpeptidase 4 inhibitors (iDPP4), and sodium-glucose co-transporter 2 inhibitors (iSGLT2) [4]. Side effect with metformin are anorexia, nausea, abdominal discomfort and diarrhoea; they are usually mild and transient. Also, metformin reduces intestinal absorption of vitamin B12.

Herbal medicine is one of the subgroups of complementary and alternative medicinal (CAM) therapies. Many patients consider CAM over conventional therapies due to dissatisfied outcomes from the conventional therapies, higher treatment costs and increased side effects of modern medicines. Therefore, the active ingredients of the medicinal plants are directing towards its particular use in diseased condition, may be applied in complex formulation of one or more plants. The use of traditional herbal medicines is more associated with patient conception and less paternalistic compared to allopathic medicine in general [5-8]. The main aim of the present study is to evaluate the anti-diabetic potential of siddha formulation Naga Chendhooram (NC) 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/XIII/127/2019.

2.2. Measurement of Oral glucose tolerance test (OGTT) [9]

Overnight fasted normal rats were randomly divided into 3 groups of each 6 animals. Group I - Animals received normal saline. Group II - Animals received 200mg/kg of NC, Group III- Animals received 400mg/kg of NC. 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 200mg/kg of NC. Animals belongs to

group IV received 45 mg/kg,i.p of STZ and treated with 400mg/kg of NC

2.4. Induction of Diabetes with Streptozotocin [10]

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 [11,12]

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 [13]

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 NC on body weight of control and STZ induced experimental rats

Body weigh measurement is become an ideal choice on prediction of efficacy of the trial drugs 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 NC 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 NC 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	223.3 ± 5.8	250.8 ± 3.71
Diabetic control (STZ 45 mg/kg,i.p)	213.8 ± 1.90	183.7 ± 4.08
STZ+200mg/kg of NC	215 ± 2.58	200.3 ± 0.95
STZ+400mg/kg of NC	221.2± 5.3	208.3 ± 4.65

Values represent mean ± SEM of 6 experimental animals.

3.2. Effect of NC 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 NC reduced the glucose level from the threshold peak level. As shown in Table 2.

Table 2: Effect of NC on Oral Glucose Tolerance Test

GROUP	Blood glucose level (mg/dl)		
	0 Min	60 min	120 min
Glucose 2 g/kg	70.5 ± 1.33	160.5 ± 1.9	120.7 ± 4.13
Glucose +200mg/kg of NC	69.33 ± 1.72	140.5 ± 2.93	132.3 ± 1.35
Glucose +400mg/kg of NC	70.5 ± 0.61	129 ± 3.88	119.5 ± 3.98

Values represent mean ± SEM of 6 experimental animals

3.3. Mean value of anemia in ante natal women maternity care visit

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 NC at both the dose level of 200 and 400 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 NC on fasting blood glucose and plasma insulin level

Group	Fasting Blood glucose level (mg/dl)			Insulin (U/L)
	0th day	14th day	28th Day	
Control	81.83 ± 3.3	81 ± 2.6	77.5 ± 2.8	17.07 ± 0.84
Diabetic control (STZ 45 mg/kg,i.p)	75.17 ± 2.91	310.5 ± 6.7	330.7 ± 6.9	5.71 ± 0.65
STZ+200 mg/kg of NC	77 ± 2.3	280.8 ± 3.0	247.2 ± 7.6	8.28 ± 0.27
STZ+400 mg/kg of NC	76.17 ± 1.9	249.7 ± 3.7	210.3 ± 4.1	9.03 ± 0.08

Values represent mean ± SEM of 6 experimental animals

3.4. Effect of NC 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 NC 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 NC on HbA1C, serum urea and serum creatinine level

Group	HbA1C (% Hb)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
Control	7.3 ± 0.60	23.5 ± 1.5	0.6 ± 0.09
Diabetic control (STZ 45 mg/kg,i.p)	13.85 ± 0.4	75.17 ± 3.6	1.783 ± 0.04
STZ+200mg/kg of NC	11.72 ± 0.69	66.17 ± 2.7	1.35 ± 0.09
STZ+400mg/kg of NC	10.28 ± 0.12	44.5 ± 1.85	0.85 ± 0.07

Values represent mean ± SEM of 6 experimental animals

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

Sample belongs to group I reveals prominent histology of islet of Langerhans and acinar zone. Degenerative fibrotic changes with invasion of inflammatory cells though connective septae and islets were observed in the sample belongs to group II. Marginal increase in integrity of islet cells with moderate signs of pyknosis were observed in sample belongs to group III rats. Increased population of isltes with distinct border surrounded by exocrine part of the pancreas were observed in sample belongs to group IV rats. As shown in Figure 1.

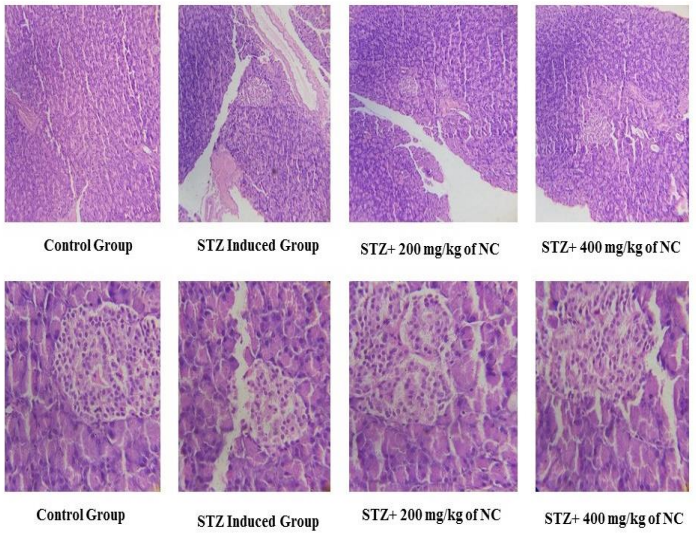

Figure 1: Histopathology of Rat Pancreas (H&E) Staining under low and high power magnification

4.Discussion

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 [14,15]. 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 [16,17]. A worldwide survey reported that the estimated incidence of diabetes and projection for year 2030 is 350 million [18].

The mechanism by which STZ destroys β-cells of the pancreas and induces hyperglycemia is still unclear. Many actions have been attributed to STZ that are similar to those that have been described for the diabetogenic action of alloxan, including damage to pancreatic β-cell membranes and depletion of intracellular nicotinamide adenine dinucleotide (NAD) in islet cells. In addition, STZ has been shown to induce DNA strand breaks and methylation in pancreatic islet cells [19,20]. Its diabetogenic action has been ascribed to an increase in the intracellular methylation reaction, DNA strand breaks, and the production of nitric oxide (NO) and free radicals.NO is involved in pancreatic destruction, where the interaction between NO and ROS modulates oxidative damage [21,22].

Despite important progress in the management of diabetes using synthetic drugs, many traditional plant treatments are still being used throughout the world [23]. Many traditional plants and herbal medicines have been found to possess the antidiabetic activity [24]; however, the World Health Organization has recommended that traditional plant treatments for diabetes warrant further evaluation [25].

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 NC at both the dose level of 200 and 400 mg/kg has remarkable reduction on blood glucose between the 14th to 28th day interval time periods. HbA1c is an established means of monitoring average blood glucose levels and a surrogate marker of the effect of glucose-lowering interventions. It is highly associated with the risk for diabetes-related complications, in particular those of microvascular origin [26]. Although HbA1c is almost universally accepted to guide and monitor diabetes treatment, its use in clinical practice has arguable limitations. There is a proposed inter-individual variation in the propensity for glycation, in both healthy individuals and those with diabetes [27]. There was a significant increase in HbA1C, Serum Urea and Creatinine level in STZ alone treated rats whereas treatment with NC at both the dose level has shown significant reversal of the above mentioned serological parameter's.

Streptozotocin (STZ) is frequently used to induce diabetes in experimental animals through its toxic effects on pancreatic β -cell [28] and as a potential inducer of oxidative stress. It has been reported that diabetes induced by STZ is the best characterized system of xenobiotic-induced diabetes and the commonly used model for the screening of anti-hyperglycemic activities [29].Sample belongs to group I reveals prominent histology of islet of Langerhans and acinar zone. Degenerative fibrotic changes with invasion of inflammatory cells though connective septae and islets were observed in the sample belongs to group II. Marginal increase in integrity of islet cells with moderate signs of pyknosis were observed in sample belongs to group III rats. Increased population of isltes with distinct border surrounded by exocrine part of the pancreas were observed in sample belongs to group IV rats.

5. Conclusion

Application of alternate therapeutic agents from the siddha origin is one of the ancient tradition, being imposed to current society in the urge to evaluate the mechanism of their underlying pharmacological action and their associated benefits and adverse effects. Thus, use of siddha drugs like Naga Chendhooram is still continued in modern society for the prevention, wellbeing and treatment of diabetes

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