Research Article

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PRE-CLINICAL EVALUATION OF ANTI-DIABETIC POTENTIAL OF MEGARI CHOORANAM IN STZ INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is a multifactorial illness characterized by impaired protein, carbohydrate, and lipid metabolism. The primary risk factors for DM include abnormalities in the lipid profile, cardiovascular, renal, and glucose metabolism. According to the WHO, almost 80% of the population uses herbal medicines to treat a variety of diseases, and they are getting increasing attention in global healthcare discussions. Megari Chooranam (MC) is a versatile siddha formulation comprises of herbs that are known for pharmacologically active therapeutics. Till date there is no documentary evidence claiming the anti-diabetic potential of this novel formulation, hence the mail aim of the present investigation is to evaluate the anti-diabetic efficacy of the siddha formulation MC in Streptozotocin (STZ) induced diabetic rats. Results of the present study projects that there was a consistent increase in glucose level of rats challenged with STZ between 14th to 28th days of experimental periods. Treatment with MC at both the dose level of 250 and 500 mg/kg has remarkable reduction on blood glucose between the 14th to 28th day interval time periodsThere was a significant increase in HbA1C, Serum Urea and Creatinine level in STZ alone treated rats whereas treatment with MC at both the dose level has shown significant reversal of the above mentioned serological parameter's. It was concluded from the results that the siddha formulation Megari Chooranam reveals promising anti-diabetic activity in the tested animal model. Hence clinical recommendation of the drug for managing diabetes will be highly appreciated.

KEY WORDS: ***Siddha, Megari Chooranam, STZ, Diabetic rats, Diabetes mellitus, Glucose level***

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1. Introduction

Diabetes mellitus (DM) is a category of chronic metabolic illnesses defined by persistently increased blood glucose levels due to insulin secretion abnormalities or insulin resistance. Diabetes now affects 382 million people globally and is projected to reach 592 million by 2035 [1]. Diabetes has become a major global health concern due to the chronic nature of the disease and its consequences, as well as the economic burden on the patient and their family members. Type 2 diabetes is an age-related condition that has become a pandemic in a number of nations [2]. Following cardiovascular disease and cancer, it is the third biggest cause of mortality [3].

The most often reported adverse effect of metformin is gastrointestinal (GI) upset, which is usually temporary and may be prevented by gradually titrating the dosage and taking the medicine with meals [4]. Pioglitazone and rosiglitazone are thiazolidinedione (TZD) medications that have been licensed by the FDA for the treatment of type 2 diabetes mellitus (T2DM). Due to the adverse effects associated with TZD, including weight gain and fluid retention, the ADA consensus statement recommends metformin over TZD as the first-line therapy for impaired glucose tolerance (IGT) or impaired fasting glucose (IFG) [5]. Herbal medicines have become a significant and necessary component of public healthcare all around the world [6]. Various assessments on traditional and alternative medicine have shown that they are widely used [7]. However, clinical research of these herbal medications should be supported in order to broaden their arena of acceptability. It is recommended that single and consistent batches of formulations be used in clinical studies to demonstrate effectiveness [8]. Although herbal practitioners and believers do not require clinical testing, it has become necessary for its widespread acceptance and survival in the worldwide market alongside modern medications [9].

The Siddha medicine pioneered the treatment of inflammation and other degenerative conditions; the majority of Siddha proration are made up of botanical substances with new therapeutic properties. Herbal supplements are well-known for their high safety index, as well as the fact that phytocomponents enhance the healing system by appropriately boosting the cellular biochemical route. Present study aimed at evaluating the anti-diabetic potential of the siddha formulation Megari Chooranam on Streptozotocin (STZ) induced diabetes in rats

2. Materials and Methods

2.1. Animals

Healthy adult Wistar albino rats of either sex weighing between 200-240 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 (Sai feeds, Bangalore, India) 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. SU/CLATR/IAEC/XVII/176/2021

2.2. Experimental Methodology

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 250mg/kg of MC. Animals belongs to group IV received 45 mg/kg,i.p of STZ and treated with 500mg/kg of MC.

2.3. Induction of Diabetes [11,12]

Streptozotocin (STZ), at a dose of 45 mg/kg body weight was dissolved in citrate buffer, injected intraperitoneally 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.

2.4. Body Weight and Glucose estimation [13]

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.

2.5. Sample Collection [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 and cardiac puncture and stored in clot activator coated test tubes for serum biochemical analysis. Pancreas sample were harvested and carefully investigated for gross lesions.

2.6. Histopathology [15]

A portion of pancreatic tissue was dissected out and fixed in 10% buffered neutral formal saline and processed. After fixation, tissues were embedded in paraffin. Fixed tissues were cut at 10 µm and stained with hematoxylin and eosin. The sections were examined under light microscope for histological changes..

2.7. Statistical Method

The statistical analysis was carried by one-way analysis of variance ANOVA. 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 MC 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 MC 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 MC 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	218.8 ± 3.188	256.3 ± 3.575
Diabetic control (STZ 45 mg/kg,i.p)	210.3 ± 1.82	177.8 ± 6.585*
STZ+250mg/kg of MC	217.2 ± 2.738	198.7 ± 5.27*
STZ+500mg/kg of MC	221.2 ± 3.146	211.7 ± 3.955*

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

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

Table 2: Effect of MC on Oral Glucose Tolerance Test

GROUP	Blood glucose level (mg/dl)		
	0 Min	60 min	120 min
Glucose 2 g/kg	67.17 ± 4.199	171.5 ± 4.28	133.8 ± 2.651
Glucose +250mg/kg of MC	72.17 ± 1.922	150.5 ± 6.657*	128.2 ± 3.516*
Glucose +500mg/kg of MC	67.83 ± 5.437	130 ± 5.298*	112.3 ± 2.14*

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

3.3. Effect of MC 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 MC at both the dose level of 250 and 500 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 MC 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	83.17 ± 2.24	82.83 ± 2.18	84.33 ± 3.41	19.15 ± 0.51
Diabetic control (STZ 45 mg/kg,i.p)	77.17 ± 3.73	328.5 ± 10.47**	348.2 ± 8.70**	5.233 ± 0.80*
STZ+250mg/kg of MC	81.83 ± 4.88	284.2 ± 6.08*	264.2 ± 3.38*	8.65 ± 0.50*
STZ+500mg/kg of MC	75.83 ± 3.28	231.8 ± 5.36*	215.8 ± 3.37*	10.2 ± 0.91*

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

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

Group	HbA1C (% Hb)	Serum Urea (mg/dl)	Serum Creatinine (mg/dl)
Control	6.817 ± 0.32	21.33 ± 1.17	0.3333 ± 0.05
Diabetic control (STZ 45 mg/kg,i.p)	14.4 ± 0.52*	78.5 ± 4.26*	1.967 ± 0.09*
STZ+250mg/kg of MC	10.8 ± 1.03*	64.33 ± 2.61*	1.533 ± 0.21*
STZ+500mg/kg of MC	10.15 ± 0.99*	40.67 ± 3.80*	1.067 ± 0.18*

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

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

Normal architecture of pancreas with densely packed acinar cells with prominent lobes were observed in sample belongs to group I. Significant pathological changes were observed in both exocrine and endocrine part of the pancreas along with swollen lobules evidenced with massive inflammatory changes were observed in the sample belongs to group II . As shown in Figure 1

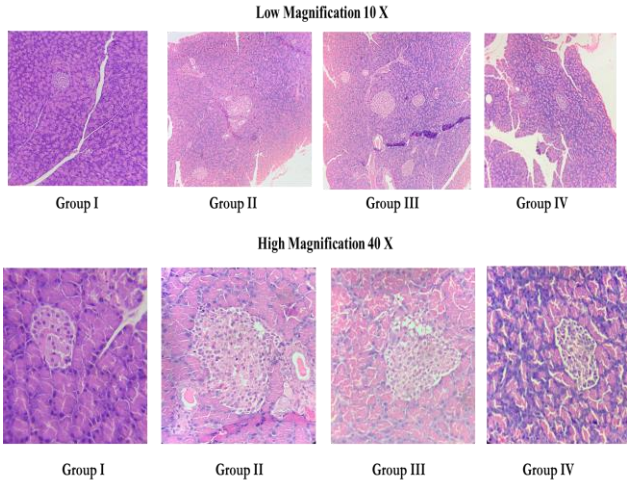

Figure 1: Histopathology of Rat Pancreas (H&E) Staining under low and high power magnification

Marginal increase in integrity and density of islet cells with moderate signs of degenerative changes were observed in sample belongs to group III rats. Significant decrease in the vacuoles formation on the acinar cells with distinct border surrounded by exocrine part of the pancreas were observed in sample belongs to group IV rats. As shown in Figure 1.

4. Discussion

Type 2 diabetes mellitus (T2DM) is the most common form of diabetes mellitus, accounting for more than 90% of all diabetes-related morbidity and death [16]. This is frequently characterized by pancreatic -cell dysfunction and insulin resistance, resulting in an increase in blood glucose [17]. Diabetes patients have benefited from the availability of antidiabetic medications such as insulin, biguanides, sulphonylureas, and -glucosidase inhibitors, among others. However, some of these medications are prohibitively expensive and not entirely accessible, particularly in poor countries, and are associated with adverse effects such as hypoglycemia, disorientation, and lactic acidosis [18,19]. These concerns fuelled a major push for the development of effective ethnomedicines, which are believed to be more affordable, accessible to diabetes patients in underdeveloped nations, and safe [20].

Streptozotocin-treated rats generated type 2 diabetes mellitus [21]. Streptozotocin causes selective damage to the pancreas's insulin-secreting -cells, resulting in diabetes. Inadequate insulin further impairs cells' capacity to utilise glucose, resulting in the formation of reactive oxygen species (ROS) [22]. Additionally, mice exhibit a variety of diabetes sequelae, including cardiomyopathy, retinopathy, nephropathy, and neuropathy, all of which are mediated by oxidative stress [23].

Secondary metabolites of plants such as alkaloids, flavonoids, tannins, phenols, saponins, and numerous other fragrant chemicals serve as a defence mechanism against invasion by various bacteria, insects, and other herbivores [24]. Flavonoids are hydroxylated phenolic substances that plants produce in reaction to microbial infection [25]. Saponin's antimicrobial function stems from its capacity to trigger protein and enzyme leaks from the cell [26]. Tannins attach to proline-rich proteins and disrupt protein synthesis [27]. These

phytocomponents' therapeutic qualities and pharmacological effects are widely established in Indian traditional medicine. Medicinal plants are recognised to include a variety of active medicinal principles and to have biological action against a variety of ailments [28].

Outcome of present study shown profound increase in the level of blood glucose level on the 60th min of the glucose challenged rats whereas treatment with MC reduced the glucose level from the threshold peak level in OGTT study. 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 MC at both the dose level of 250 and 500 mg/kg has remarkable reduction on blood glucose between the 14th to 28th day interval time periods.

5.Conclusion

Diabetes mellitus (DM) is a collection of metabolic abnormalities that impact not just glucose metabolism but also protein and lipid metabolism. According to a recent assessment on the global incidence of diabetes, 463 million individuals are now affected by the disease. Due to increasing side effects caused by the conventional anti-diabetic agents it's a time to explore the safe therapeutics from alternate drug of choice. It was concluded from the results that the siddha formulation Megari Chooranam reveals promising anti-diabetic activity in the tested animal model. Hence clinical recommendation of the drug for managing diabetes will be highly appreciated.

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