Research Article

# Hypoglycemic Activity of Seerankottai Thiravam (*Semicarpus Anacardium. Linn)* in Alloxan Induced Diabetic Rats

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**ABSTRACT**

Diabetes is a global disease. Entire population of the world is suffering from the disease. The International Diabetes Federation (IDF) has estimated that 366 million of people have diabetes in 2011; by 2030 it has been raised up to 552 million. It estimated that 40 million people with diabetes in India in 2007 and this may predicted to rise to almost 70 million by 2025. The aim of the study was to evaluate the anti diabetic activity of the siddha formulary medicine Seerankottai thiravagam (*Semicarpus anacardium Linn*) (SKT) in Alloxan induced rats. The diluted form of thiravagam (1 ml/kg, p.o) was administered continuously for 14 days orally. Then the results were compared with the standard drug Glibenclamide (5 ml/kg). After 5 days treated with Seerankottai thiravagam significantly ($p<0.05$) decreased fasting blood serum glucose level in diabetic rats. Treatment with Serankottai Thiravagam not significantly enhanced the average body weights of rats which indicate muscle wasting resulted due to hyperglycaemic condition. The possible mechanism for this action might be due to the inhibition of the enzyme glycogen phosphorylase, an enzyme that catalyzes the process of glycogenolysis. This might be the cause for depletion of glucose and lipid parameters such as total cholesterol and triglyceride in hyperglycaemic condition. Thus the claim made by the traditional Indian siddha systems of medicine regarding the use of Serankottai Thiravagam in the treatment of diabetes stands confirms.

**Keywords:** Alloxan induced diabetic rats, hypogycemic activity, seerankottai thiravagam,

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## INTRODUCTION

Diabetes is a global disease. Entire population of the world is suffering from the disease. The International Diabetes Federation (IDF) has estimated that 366 million of people have diabetes in 2011; by 2030 it has been raised up to 552 million. It estimated that 40 million people with diabetes in India in 2007 and this may predicted to rise to almost 70 million by 2025. It is estimated that every fifth person with diabetes will be an Indian. This indicates presence of Genetic basis for Type2 diabetes in ethnic Indian population. WHO estimates that diabetes, heart disease and stroke together will cost about $333.6 billion over the next 10 years in India alone (1). All the symptoms of Diabetes mellitus are analogous with Neerizhivu in the text, Siddha maruthuvam (pothu) (2). The siddha drug Serankottai (*Semicarpus anacardium*) has been tried for many skin diseases not for the metabolic disease like diabetes. Yagopu vaithiya chinthamani -700 describes a preparation quoted as Serankotai thiravagam (SKT) (3) is therapeutically preferred for diabetes. Being the sasthric preparation its efficacy seldom proved. The moderate anti diabetic activity of SKT is proved in preclinical studies done in Alloxan induced diabetic rats.

## MATERIALS AND METHODS

### Animals

Wistar albino rats (8–10 weeks) of both sexes were obtained from the animal house of School of Pharmacy, Vels University, Chennai. Before and during the experiment, rats were fed with standard diet (Sai durga

foods, Bangalore). After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature, relative humidity, and dark/light cycle (4). Animals described as fasting were deprived of food and water for 16 hours ad libitum. The present study was conducted after getting experimental protocol approval from Institutional Animal Ethics Committee (IAEC). (No. XIII/ VELS/PCOL/06/2000/ CPCSEA/IAEC/ 08.08.2012)

Drugs, Chemicals and stock solution Alloxan (Loba chemie, Mumbai, India), and diagnostic kits (Biolab diagnostics, Mumbai, India) were used in this study. Other chemicals used were of analytical grade and were obtained from local suppliers. The drug Serankottai Thiravagam was diluted with saline. The drug was administered continuously for 21 days orally using an oral feeding tube. The results were compared with that of the standard drug Glibenclamide which was also given continuously for 21 days.

**Fig 1: Purified Seerankottai (Semicarpus Anacardium)**

**Oral Glucose Tolerance Test**

Rats were divided into five groups containing six animals in each group. All animals fasted before treatment. Group I was kept as vehicle control which received only saline p.o., group II received glucose only (2g/kg, p.o.), group III received Serankottai Thiravagam 1ml/kg, group IV received Serankottai Thiravagam 2ml/kg. The rats of group V were treated with Glibenclamide. Blood samples were collected by puncturing the retro orbital sinus just prior to drug administration, and 30, 90 minutes after loading glucose. Serum glucose level was measured immediately (4).

**Acute Oral Toxicity Studies**

Acute oral toxicity study was performed as per OECD-425 guidelines. Mice (n = 6) of either sex selected by random sampling technique were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the Serankottai Thiravagam in normal saline was administered orally at the different dose levels in up and down dosing schedule according to body weight by gastric intubation and observed for 14 days (5).

**Experimental Design**

Five groups of rats, six in each received the following treatment schedule.

Group I: Normal control (saline).

Group II: Alloxan treated control (150mg/kg.ip).Group III: Alloxan (150mg/kg.i.p) + Serankottai Thiravagam 1ml/kg, p.o,

Group IV: Alloxan (150mg/kg.ip) + Serankottai Thiravagam 2ml/kg, p.o

Group V: Alloxan (150mg/kg.ip) + Standard drug, Glibenclamide (5 mg/kg, p.o).

Serankottai Thiravagam and standard drug glibenclamide (5mg/kg) and saline were administered with the help of feeding

cannula. Group I serve as normal control, which received saline for 14 days. Group II to Group V are diabetic control rats. Group III to Group V (which previously received alloxan) are given a fixed dose Serankottai Thiravagam (1ml/kg, p.o), (2ml/kg, p.o) and standard drug glibenclamide (5mg/kg) for 14 consecutive days (6).

**Induction of Diabetes in Experimental Animals**

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg). Alloxan was first weighed individually for each animal according to the body weight and then solubilized with 0.2 ml saline just prior to injection. Two days after alloxan injection, rats with plasma glucose levels of >150 mg/dl were included in the study. Treatment with Serankottai Thiravagam was started 48 h after alloxan injection.

**Collection of Blood Sample and Blood Glucose Determination**

Blood samples were drawn from tail tip of rat at weekly intervals till the end of study. Fasting blood glucose estimation and body weight measurement were done on day 1, 7, and 14 of the study. Blood glucose estimation was done by one touch electronic glucometer using glucose test strips. On day 14, blood was collected from retro-orbital plexus under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated (7).

Serum was separated and analyzed for serum cholesterol, serum triglycerides, serum HDL, serum LDL was estimated. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formalin solution, and immediately processed by the paraffin technique. Sections of 5$\mu$ thickness were cut and stained by haematoxylin and eosin for histological examination.

**Statistical Analysis**

All the values of body weight, fasting blood sugar, and biochemical estimations were expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and Dunnet's *t*-test. Differences between groups were considered significant at $P < 0.01$.

**RESULTS AND DISCUSSION**

The OECD guidelines AOT-425 was followed for estimation of acute toxicity study in mice. Mortality in the acute oral toxicity test was seen in the dose 2 ml/kg. The animals showed severe toxic signs and abnormal behaviour like itching, restlessness and aggressiveness etc. Hence, as per the siddha literature recommendations the main stock was further diluted ten times with saline and from this diluted stock solution 1ml and 2 ml/kg dose was considered for the further pharmacological evaluation. To ascertain a scientific base for the usefulness of this drug in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following glucose tolerance test and the alloxan-induced model (**Table 1**).

After alloxon administration, there was severe hyperglycaemia in all the animals when compared with the normal animals. The upper bound dose 2 ml/kg doses of Serankottai Thiravagam significantly lowered the elevated blood glucose level when compared with that of diabetic control. It was observed that the standard drug glibenclamide lowered the blood glucose level significantly bringing it nearly back to normal, whereas Serankottai Thiravagam significantly ($P<0.05$) decreased fasting blood serum glucose in the diabetic rats after five days of treatment compared with the initial blood serum glucose levels (**Table 3**). However, the sugar control effect of the Serankottai Thiravagam was incomparable to that of the reference drug. In the present investigation, statistical analysis revealed that the 21 days treatment with standard drug Glibenclamide showed significant decrease in glucose, cholesterol, triglyceride VLDL, LDL and increase in body weight (**Table 2**) and HDL level (**Table 4**), thereby exhibited significant antidiabetic activity.

Alloxan, a beta cytotoxin, induces 'chemical diabetes' in a wide variety of animal species by damaging the insulin-secreting cells of the pancreas. Though not routinely used anymore, the oral glucose tolerance test is the gold standard for making the diagnosis of type 2 diabetes. It is still commonly used for diagnosing gestational diabetes also. Literature sources indicate that alloxan treated rats are hyperglycemic. The use of alloxan (150 mg/kg b.w.) produced a partial destruction of pancreatic β-cells even

though the animals became permanently diabetic. Thus, these animals have surviving β -cells and regeneration is possible. The acute oral toxicity study of Serankottai Thiravagam showed mortality at 2ml/kg. Since the Serankottai Thiravagam was identified with remarkable toxicity at the higher dose in the acute toxicity study. Hence the stability and tolerance was observed at 0.2 ml/kg dose level.

Administration of diabetogenic agent alloxan 150mg/kg, i.p. lead to elevation of fasting blood glucose levels, which was maintained over a period of 2 weeks. Alloxan caused body weight reduction (P<0.01), which is reversed by Serankottai Thiravagam at the dose (2 ml/kg) is more effectively after 5days of treatment.

The control rats had the blood glucose level 74.52 ± 2.42 mg/dl while untreated diabetic rats showed 215.72±10.14mg/dl blood glucose level. On day 5, 10 and 14 of treatment at 2ml dose of Serankottai Thiravagam reduced the blood glucose level to 184.40±10.26, 172.14±8.65 and 158.00±9.00 mg/dl (P<0.01) respectively. In present investigation, it was observed that Serankottai Thiravagam can reverse the effects of Alloxan induced diabetes to a significant level.

**Histopathological changes in pancreas:**

**Normal** - Normal acini and normal cellular population in the islets of Langerhans in pancreas of normal untreated rats.

**Diabetic Control**- Severe damage to the islets of Langerhans and reduced dimensions of islets results damage of pancreas in alloxan-treated diabetic control rats.

**Serankottai Thiravagam 1 ml/kg**-The moderate damage to the islets of Langerhans and reduced dimensions of islets.

**Serankottai Thiravagam 2 ml/kg**- partial restoration of normal cellular population and enlarged size of *β*-cells with hyperplasia were seen. **Standard-** Restoration of normal cellular population size of islets with hyperplasia by Glibenclamide was seen.

**Table 1: Oral glucose tolerance test**

| Treatment ( dose/ kg body weight) | Blood glucose (mg/dl) Fasting | 30 min | 90 min |
|---|---|---|---|
| **Normal** | 72.4 ± 2.4 | 81.20 ± 2.1** | 86.4 ± 2.8** |
| **Glucose; 2 g.** | 72.9 ± 2.6 | 168.24 ± 1.8 | 226.04 ± 6.4 |
| **SKT (1 ml/kg)+Glucose** | 74.1 ± 2.5 | 96.20 ± 3.4** | 90.12 ± 4.2** |
| **SKT-II (2 ml/kg)+Glucose** | 73.6 ± 2.2 | 84.42 ± 2.32** | 81.00 ± 2.3** |
| **Glibenclamide (5 mg/kg)** | 71.5 ± 2.4 | 96.18 ± 4.12** | 92.15 ± 6.1** |

Values are as mean ± S.E.M **P <0.01; Vs group II; n=6

**Table 2: Measurement of body weight changes after Serankottai Thiravagam treatment**

| Drug treatment | Periodical weight changes Day 0 | Day 1 | Day 2 | Day 4 | Day 8 | Day 14 |
|---|---|---|---|---|---|---|
| **Normal** | 158.32± 2.12 | 161.30± 3.00 | 163.15± 2.52[b],* | 164.88± 3.45[a],** | 168.22± 4.18[a],** | 172.70± 4.18[a,],** |
| **Diabetic control** | 160.41± 2.16 | 156.72± 2.42 | 152.8± 2.20 | 148.11± 2.33 | 122.34± 2.12 | 113.40± 3.20 |
| **SKT -I 1ml/kg** | 158.67± 2.44 | 160.12± 2.30 | 158.82± 2.54 | 155.28± 4.63 | 141.00± 3.15[a,]** | 133.12± 3.24[a,]** |
| **SKT -II 2ml/kg** | 159.52± 2.50 | 161.40± 2.25 | 157.17± 3.28 | 143.20± 3.46[a] | 133.72± 3.37[a] | 121.10± 2.30[a] |
| **Glibenclamide (5 mg/kg)** | 158.45± 2.34 | 161.76± 2.58 | 164.42± 2.71* | 170.16± 2.28** | 172.53± 2.93** | 182.12± 2.95** |

Values are as mean ± S.E.M [aP] <0.001; [b]P <0.05 Vs Normal, **P <0.01; *P <0.05 Vs Diabetic Control; n=6

**Table 3: Fasting Serum Glucose Concentration is Normal and Alloxan-Induced Diabetic Rats**

| Treatment | Fasting serum Glucose concentration (mg/dl) measured at regular intervals | | | |
|---|---|---|---|---|
| | Day 1 | Day 5 | Day 10 | Day 14 |
| **Normal** | 74.52 ± 2.42[a,**] | 72.37 ± 3.04[a,**] | 74.30 ± 4.10[a,**] | 72.23±2.32[a,**] |
| **Diabetic control** | 215.72± 10.14 | 226.14 ± 11.4 | 254.25 ±9.12 | 292.56±12.88 |
| **SKT -I 1ml/kg** | 211.25 ± 8.02[a] | 196.22 ± 12.13[a] | 182.72 ± 10.00[a,**] | 180.21±11.32[a,**] |
| **SKT -II 2ml/kg** | 222.18 ± 9.24[a] | 184.40 ± 10.26[a,*] | 172.14 ± 8.65[a,**] | 158.00±9.00[a,**] |
| **Glibenclamide (5 mg/kg)** | 230.62 ± 10.11[a] | 162.12±8.21[a,**] | 158.4 ± 9.14[a,**] | 128.65±7.16[a,**] |

Values are as mean ± S.E.M [aP] <0.001; [b]P <0.05 Vs Normal, **P <0.01; *P <0.05 Vs Diabetic Control; n=6

**Table: 4: Lipid Profile In Normal and Effect of Serankottai Thiravagam In Alloxan-Induced Diabetic Rats**

| Treatment | Dose | Parameters (mg/dl) | | | |
|---|---|---|---|---|---|
| | | Total Cholesterol | Triglycerides | HDL | LDL |
| **Normal control** | 10 mg/kg of vehicle | 75.60±9.00 | 70.46±5.88 | 35.10±8.72 | 36.22±2.16 [a,**] |
| **Diabetic control** | - | 82.10±8.92 | 74.20±10.22 | 27.02±2.28 | 138.14±5.21 |
| **SKT –I** | (1 ml/kg) | 79.01±6.46 | 80.62±9.12 | 36.81±5.62 | 79.92±5.19 [a,**] |
| **SKT –II** | (2 ml/kg) | 72.18±5.81 | 74.40±6.51 | 34.00±3.13 | 68.10±5.45 [a,**] |
| **Glibenclamide** | (5 mg/kg) | 84.2±6.22 | 72.51±7.09 | 40.52±10.00 | 34.18±5.10 |
| **Normal control** | 10 mg/kg of vehicle | 75.60±9.00 | 70.46±5.88 | 35.10±8.72 | 36.22±2.16 [a,**] |

Values are as mean ± S.E.M; [a]P <0.001; [b]P<0.01; [c]P<0.05 Vs Normal; [d]P <0.001 Vs Diabetic; n=6

## CONCLUSION

This study results indicates that Serankottai Thiravagam have significant anti-hyperglycaemic activities in alloxan-induced hyperglycaemic rats with changes in body weight. Hence the above discussion reveals that Serankottai Thiravagam at high dose (2ml/kg) is moderately effective. The knowledge of the system of diabetes mellitus, as the history reveals, existed with the Indians since prehistoric age. “NEERIZHIVU”' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body, i.e., in sweat, mucus, breathe, blood, etc. In the present study, diabetic rats had lower body weights, high blood glucose level as compared to the normal rats.

Treatment with Serankottai Thiravagam not significantly enhanced the average body weights of rats which indicate muscle wasting resulted due to hyperglycaemic condition. So it can be concluded that the Serankottai Thiravagam have moderate anti diabetic effects in alloxan-induced diabetic rats. The possible mechanism for this action might be due to the inhibition of the enzyme glycogen phosphorylase, an enzyme that catalyzes the process of glycogenolysis. This might be the cause for depletion of glucose and lipid parameters such as total cholesterol and triglyceride in hyperglycaemic condition. Thus the claim made by the traditional Indian siddha systems of medicine regarding the use of Serankottai Thiravagam in the treatment of diabetes stands confirms.

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