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ANTIDIABETIC ACTIVITY OF *KARCHURE CHOORANAM* ON ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Phoenix dactylifera linn (*Arecaceae*) commonly known as Date palm has been widely used in Siddha system of medicine for diabetes. The powder form of dried dates (*Karchure chooranam*) showed a significant inhibitory effect was screened at 500mg/kg, for the in vivo anti-diabetic activity on alloxan induced diabetic rats. Glibenclamide (10mg/kg) used as reference standard. Single dose (500 mg/kg) treatment with the siddha drug Karchure chooranam produced 69.34% anti-hyperglycaemic effect (antidiabetic effect). The trial drug showed a significant anti-diabetic activity ($P<0.01$) and were comparable with that of standard thus validating the traditional claim of the plant.

KEYWORDS: *Phoenix dactylifera linn*, Siddha system, Glibenclamide, Anti-diabetic activity, Alloxan

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INTRODUCTION

Diabetes mellitus is a debilitating and often life-threatening metabolic disorder with increasing incidence throughout the world. Diabetic complications arise partly from glycosylation damage to structural and functional proteins and reflect chronic failure to maintain blood glucose homeostasis. Other complications such as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and diabetic cardiomyopathy prevail as a result of hyperglycemia. Non-insulin dependent diabetes mellitus accounts for over 85% of diabetes mellitus worldwide and is associated with a high incidence of morbidity and mortality.[1] Thereis an estimated 143 million people worldwide suffering from the disease and this is almost five times the estimate ten years ago. It has been predicted that the number may probably double by the year 2030. With an estimated 50.8 million people living with diabetes, India has the world's largest diabetes population, followed by China with 43.2 million. [2]The introduction of Insulin and later oral hypoglycaemic agents, revolutionized the management of diabetes mellitus inspiteof advances in drug management of diabetes, there are still complications and adverse drug reactions.[3]
The Siddha medicine is deep rooted and it was a well branched system of medicine which gives shelter of good health to Tamil people and the countries which were attached with them for trade. The herbals which are used in our Siddha system were selected by the Siddhar's only after their great knowledge of taste, sub taste and its divisions and bio-transformation. By this outstanding knowledge and the prophetical power, they want to develop the healthy mankind without any illness, (both physical and mental wellbeing) what nowadays WHO wants to do in this whole world. Date Palms are dioecious; i.e. the male and female parts are on separate plants. The Date Palm is the tallest of the Phoenix species growing to 30m in some places. The trunk, in cultivation, is surrounded from the ground upward in a spiral pattern of leaf bases. The leaves are large, 4-5m, alternate, sheathing in dense terminal rosettes pinnately lobed. The fruit of the date palm is well known as a staple food composing a fleshy pericarp and seed. The date seeds (pits) constitute approximately 10% of the fruits. Dates is a high-energy food, being high in carbohydrates (70-80%) and low fats and proteins, and a good source of vitamin, calcium, magnesium, phosphorous, zinc, iron, potassium, tannins, flavonoids, phenolic compounds and iodine.[4] The dry dates are having low glycaemic index as 65-70. [5]In Siddha, this disease was classified under one of the 21 types of increased micturition diseases (Neerinai perukkal noigal) as Mathu megam or inippu neer (sweet urine), what exactly the Greek word diabetes mellitus also means. Siddha system has a wide range of herbal drug for diabetes mellitus. The herbal drug Phoenix dactylifera is easily available, less cost and a very good nutraceuticals.

MATERIALS AND METHODS

(i) Animals

Albino rats (Wistar strain) of either sex weighing 210–230gm were used. Animals were fed on conventional diets and water *ad libitum* and they were maintained under standard conditions of humidity, temperature (20-24°C) and light (12 h light: 12 h dark cycle).They were fasted for about 18 h prior to the experiment, with access to water. Animals were kept in polycarbonate cages with laced steel roofs. The animals were acclimatized for one week under laboratory conditions. The study was conducted at the Vel's University, Chennai after obtaining Institutional Animals Ethical Committee clearance bearing the number (XII/ VELS/ COL/02/ CPCSEA/ IAEC/ 23.09.11).

(ii) Drugs Collection and Authentication

The dried unripe date fruits used in this study were procured from TAMPCOL outlet store, Arumbakkam and fruit market, Chennai. The trial drug was identified from the sample preserved in the Pharmacology department and it was authenticated by the Botanist, (Dept. of Pharmacognosy), CRRI Siddha Arumbakkam, Chennai-600106.

(iii) Preparation of the Trial drug

The best among the purchased raw drug were selected, shadow dried, pulverized and sieved to get a fine powder. The purification process for the chooranam is depends on nature and active principles of the chooranam, because the trial drug contains Phenolic compounds which are easily denatured by heating. Then this chooranam was stored in a closed and air tight container. Every time the needed medicine for the trial was prepared in this same fashion. Because the life period of the Chooranam is only three months, the prepared Chooranam was used within the stipulated period. The trial drug was kept in Pharmacology department for further research work.

(iv) Acute Toxicity Study

The albino mice weighing between 22-28 gm were selected to ascertain the toxicity range of the test drug *Karchure chooranam*. The starting dose administered to the test group of animals was 500mg/kg. The animals were segregated into six groups consisting of six mice each. The increasing doses were administered up to 5000mg/kg. The toxic dose was determined by observing the mortality rate in the drug treated groups. From this the therapeutic dose was selected for the further study.[6]

(v) Antidiabetic evaluation

The normal and healthy rats were used to induce diabetes after acclimatization period of 10 days. These animals were injected with freshly prepared aqueous solution of alloxan monohydrate (Sigma chemical company, USA) at a dose of 100mg/kg by intraperitoneal route.10% dextrose was there after administered orally to combat the immediate hypoglycaemia that could occur. The serum glucose was observed 48h after alloxan administration. The serum glucose levels were observed for 7 days once. The rats showed serum glucose level ranging 220-270 mg/dl. Animals were fed with the same standard diet and water ad libitum. They were fasted for about 18 h prior to the experiment providing access to water. The drug *Karchure chooranam* was administered at dose level of 500mg/kg body weight orally to the group containing 6 rats. Blood samples were collected as mentioned earlier.[7]

(vi) Blood collection and analysis

For the estimation of blood glucose, cholesterol and triglyceride, the blood samples were collected by orbital sinus puncture under mild ether anaesthesia in Eppendroff's tubes (1 ml) containing 50 µl of anticoagulant (10% trisodium citrate) and plasma was separated by centrifuging at 6000 rpm for 15 min and estimated in UV/Vis Spectrophotometer (Shimadzu). The absorbance of the sample and the standard was measured against the reagent blank at 500 nm. The sample solution was prepared by adding 10 µl of the plasma, reagent blank and the standard solution was prepared by adding 10 µl of the standard (Glucose, CHL and TGL) with 1000 µl of the reagent blank. The values are expressed as mg/dl. Concentration of the sample = Absorb of sample × Conc of Std/Absorb of Std

(vii) Histopathology of Pancreas

At the end of the drug treatment and after blood collection the animals from each group was sacrificed with the help of diethyl ether euthanasia method and the abdomen was cut opened and the pancreas was carefully isolated and fixed in 10% formalin solution. The pancreas was embedded in paraffin and sectioned and stained with hematoxylin and eosin and were examined microscopically for histopathological changes.[8]

(viii) Statistical analysis

The quantitative measurements in all the experiments were made on 6 animals in each group and the values are expressed as MEAN±SEM. The data were subjected to one-way ANOVA to determine the significance of changes followed by Dunnett's multiple comparisons to analysis the significance of difference within the experimental groups. Where $P<0.05$ were taken as significant.

RESULTS AND DISCUSSION

The traditional system of medicine became significantly more popular because of the curative property, less toxic and has no side effects. [9]Siddha system of medicine (SSM) is

one such ancient traditional system of India. [10,11]From the acute toxicity study was performed as per OECD guideline-425, it was concluded that the test drug *Karchure chooranam* has no lethal effect upto 5gm/kg after oral administration in mice. Hence, as per the literature guidelines 1/10th of the dose was fixed to evaluate the antidiabetic activity of *Karchure chooranam*.

Table 1
Serum Glucose concentration in normal and Alloxan-induced diabetic rats after Karchure chooranam

Treatment	Fasting serum Glucose concentration (mg/dl) measured at regular intervals			
	Day7	Day14	Day21	Day28
Normal Group	76.22±2.41	72.83±1.24	68.33±2.66	74.25±1.45
Diabetic Control	298.33±8.2	310.33±13.26	324.33±15.12	345.60±15.18
Karchure chooranam (500mg/kg)	242±6.22**	250.27±10.28*	202.16±8.66**	179.48±6.52**
Glibenclamide (10 mg/kg)	79.34±2.74**	94.66±2.76**	95.66±2.58**	106.04±2.27**

Where n=6; Values are expressed as MEAN±SEM; *P <0.05; **P <0.01 Vs Diabetic Control

Blood sugar level was increased as expected in alloxan-injected animals, since alloxan causes a massive reduction in insulin release by the destruction of the β-cells of the islets of Langerhans and inducing hyperglycemia. Oral administration of *Karchure chooranam* at 500 mg/kg resulted in a significant reduction in the blood glucose levels. (P<0.01) The number of functionally intact β-cells in the islet organ is of decisive importance to the development course and outcome of diabetes. The total β-cell mass reflects the balance between the renewal and loss of these cells. Glibenclamide is standard drug causes decrease in blood glucose 106.04 mg/dl on 28th day while the *Karchure chooranam* also decrease the blood glucose 219.48 on 28th day. The comparative results on percentage serum glucose reduction in normal rats and after alloxan treated diabetic rats were estimated. Evidence has been accumulated in the past few years supporting that diabetes was precipitated by stress. Additionally, it was also reported that hyperglycaemia itself increases stress. The diabetes was induced with alloxan, since it was more economical and easily available. Moreover, alloxan was reported to produce diabetes by damaging pancreas by free radical related mechanisms. Rat was used since it was routinely used animal model for quick screening of drugs for their hypoglycaemic/antihyperglycemic action. Since small amount of blood was required for glucose analysis, the blood samples were collected by retro-orbital puncture as it was reported to be good method when small samples of blood were required. Currently available treatment is far from satisfactory and is expensive.

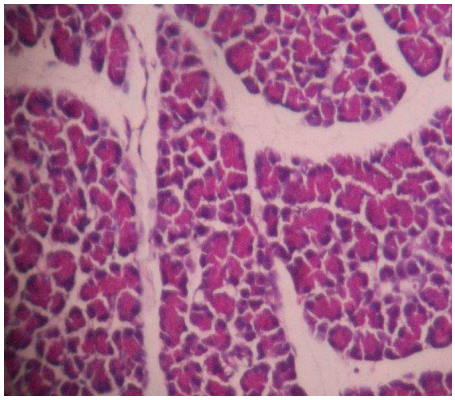

Figure-1: Normal

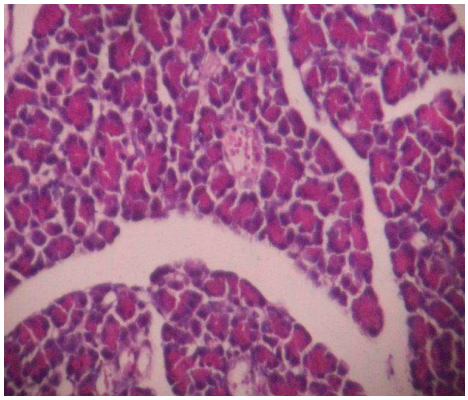

Figure-2: Control

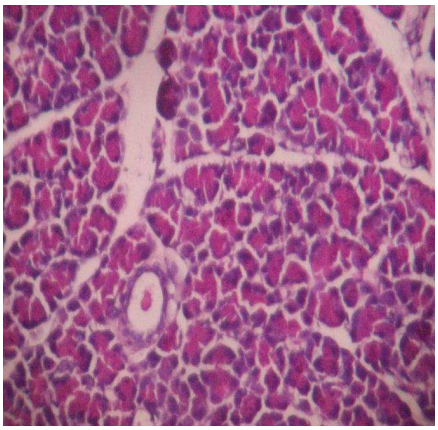

Figure-3: *Karchure chooranam*

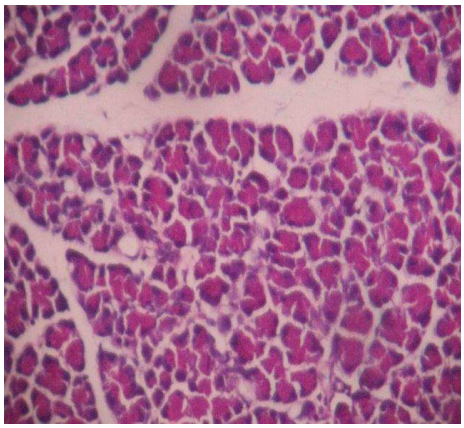

Figure-4: Glibenclamide

The histopathological report of normal rats reveals that the pancreas with acini and normal islets. Control shows atrophic with degreased islets (atrophic and small islets). *Karchure chooranam* and Glibenclamide treated group shows pancreas with acini and normal islets. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world. In spite of the introduction of hypoglycaemic agents, diabetes and the related complications continue to be a major medical problem.

CONCLUSION

According to the standard working protocol, 28days daily treatment with test drug *Karchure chooranam* significantly reduced the elevated blood glucose in alloxan induced diabetic rats while it had no effect on blood glucose of normal rats. The anti-diabetic activity of *Karchure chooranam* may be attributed to the active ingredients present in the drug. With alloxan treatment the blood glucose was raised and it was in the range of 220-270 mg/dl in different rats after stabilization period of 15days. Single dose (500 mg/kg body weight, oral) treatment with the siddha drug *Karchure chooranam* produced 69.34% antihyperglycemic effect (antidiabetic effect). Finally it can be concluded that *Karchure chooranam* (*Phoenix dactylifera*) was found to possess remarkable ($P<0.01$) anti-diabetic action in alloxan induced diabetic rats.

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