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ANTIDIABETIC ACTIVITY OF KOVAI KIZHANGU CHOORANAM IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Kovai Kizhangu Choornam has been widely used in Siddha system of medicine for diabetes. The powder (*Kovai Kizhangu Choornam*) showed a significant inhibitory effect was screened at 500 mg/kg, for the in vivo anti-diabetic activity on alloxan induced diabetic rats. Glibenclamide: Metformin (0.5:40mg/kg) used as reference standard. Single dose (400 mg/kg) treatment with the siddha drug *Kovai Kizhangu Choornam* produced anti-hyperglycaemic effect (antidiabetic effect). The trial drug showed a significant anti-diabetic activity and were comparable with that of standard thus validating the traditional claim of the plant.

Keywords: *Kovai Kizhangu Choornam*, Siddha system, Glibenclamide, Metformin, Anti-diabetic activity, Alloxan

INTRODUCTION

Diabetes mellitus (Madhumegam) was known to ancient Indian Physicians and an elaborate description of its clinical features have been described in siddha system of medicine. A number of herbs have been known to possess anti-diabetic properties. Non-insulin dependent diabetes mellitus accounts for over 85% of diabetes mellitus worldwide and is associated with a high incidence of morbidity and mortality, the contributing factors being microvascular, macrovascular neuropathy complications. Diabetes mellitus is the common metabolic disorder charecterised by hyperglycaemia. There are 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. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes. The number of individuals afflicted with diabetes mellitus will increase at a faster pace in future. Increasing knowledge of etiopathological mechanisms has provided new horizons in the management of diabetes mellitus. Experimental and clinical evidences suggest that correlation exists between the severity of microvascular and the degree of metabolic control. The ideal treatment of diabetes would allow the patients to lead a normal life in addition to achieving a normal or near normal metabolic state to avert long-term complications of diabetes mellitus. The introduction of Insulin and later oral hypoglycaemic agents, revolutionized the management of diabetes mellitus in spite of advances in drug management of diabetes, there are still complications and adverse drug reactions. None of them were unequivocally successful in maintaining normal blood glucose levels and in avoiding complications. In spite of all the advances

in therapeutics, diabetes still remains a major cause of morbidity and mortality in the world. Aside from drugs, diet control also plays a key role for the management of diabetes. Foods of high fiber content like fruits, vegetables, grains, beans and foods that contain less amount of fat, cholesterol, sugar and salt are useful in maintaining normal glucose levels in diabetic patients.

It has also been shown that alloxan induces its diabetogenic activity mainly by inducing the formation of oxygen free radicals and thereby damaging the pancreas. Present therapeutic strategies mostly try to relieve the clinical manifestation of diabetes and complications. Since diabetes seems to be a stress - related disorder. It is amazing to note that volumes of books have been written on diabetes and thousands of scientists are carrying out research in this field .yet the proportion of diabetes achieving good metabolic control is relatively small. Since more than $3/4^{th}$ of diabetic patients are managed by physicians to accomplish this goal, primary physicians need to play control role. Moreover in the due course of diabetes there may appear diabetic and or other diseases. As time elapses new techniques, both medical and educational may have been developed. This necessitates the provision of new information and acquisition of new skills, which should help to the better management of diabetes. Continuing education of patients may serve a variety of purposes. It helps the patients in achieving improvement in metabolic control. Patients gain confidence and develop the positive perception of empowerment for diabetes management and control, greater social interest and a more flexible lifestyle. It also serves to provide moral and emotional support and may facilitate the development of new coping skills, thus offering a valuable contribution to the patient's quality of life. Consequently, in the long term, this may result in decreased likelihood of late complications or better management of those complications, which exists. In recent years, siddha drugs are being effectively tried in a variety of patho physiological states. Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. It represents a heterogeneous group of disorder having hyperglycemia, which is due to impaired carbohydrate (glucose) utilization resulting from a deficient insulin secretory response. Along with hyperglycemia and abnormalities in serum lipids diabetes is appreciated with micro and macro vascular complications, which are the major causes of morbidity and death in diabetic subjects.

To date there are different groups of oral hypoglycaemic agents for chemical use, having characteristic profiles of side effects. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with anti-diabetic activity and less side effects. The objective of the study was to evaluate the siddha drug Kovai Kizhangu chooranam for hypoglycaemic and antidiabetic activities in normal and diabetic rats respectively.

MATERIALS AND METHODS

Acute toxicity study

The albino mice weighing between 22-28gms were selected to ascertain the toxicity range of the test drug *Kovai Kizhangu Chooranam*. The starting dose administered to the test group of animals was 100mg/kg. The animals were segregated into six groups consisting of six mice each. The increasing doses were administered upto 4000mg/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.

Animals

Albino rats (Wistar strain) of either sex weighing 200–300 g procured from Animal housing facility of Vel's college were used in the study. Animals were divided into six groups each containing five and were provided with standard Pellet diet and water *ad libitum*. They were fasted for about 18 h prior to the experiment, with access to water. During the experiment, water was removed while fasting was continued. The drug was administered at dose level of 400mg/kg body weight orally to a group containing 5 animals. A control group was

maintained simultaneously and received distilled water orally. Blood samples were collected from retro-orbital plexus of each rat before and at 2, 4, 6 and 8 h after test drug administration and were analyzed for glucose content.

Preparation of the drug solution

The drug solution was prepared by dissolving the drug in distilled water.

Studies in diabetic rats: Induction of diabetes

The same groups used earlier (normal) were used to induce diabetes after a washout period of 10 days. These animals were injected with freshly prepared aqueous solution of alloxan monohydrate (Sigma chemical company, USA) at a dose of 100 mg/kg body weight by intraperitoneal route.10% dextrose was there after administered orally to combat the immediate hypoglycaemia that could occur. The serum glucose was observed 20 h after alloxanisation. The serum glucose levels were observed daily for 5 days. The rats showed serum glucose level ranging 280 - 340 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 was administered at dose level of 400mg/kg body weight orally to the group containing 6 rats. The control group received distilled water only. Blood samples were collected and analyzed as mentioned earlier.

Data and statistical analysis

Data were expressed as Mean ± Standard Error of Mean (SEM). The significance of blood glucose reduction produced by the drug compared to normal and diabetic control was determined by applying ONE WAY ANOVA followed by Dunnet's multiple comparison test. P values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

From the acute toxicity study, I have concluded that the test drug *Kovai Kizhangu Chooranam* has no lethal effect upto 4g/kg body weight after oral administration in mice. Hence, as per the literature guidelines $1/10^{th}$ of the dose was fixed to evaluate the anti diabetic activity of *Kovai Kizhangu Chooranam.* The comparative results of % serum glucose reduction with test drug in normal rats were given in Table 1. The comparative results of % serum glucose reduction with alloxan induced diabetic rats were given in Table 2. 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.

Table 1 ***Oral glucose tolerance test***

Treatment (Dose / kg body weight)	Blood glucose (mg/dl)		
	Fasting	30 min	90 min
Normal control	80.5±1.80	158±8.96	233.5±3.92
KKC (400mg/kg)+Glucose	74.16±1.759*	89.33±2.679**	213±8.06*
Glibenclamide/Metformin (0.5:40mg/kg)	84.16±1.64^{ns}	96.33±2.894**	84.83±1.57**
Zinc Insulin (4 U)	84.66±1.145^{ns}	89.83±2.242**	86.5±1.258**

Values are expressed as mean ± S.E.M.; n = 6

Table.2 ***Measurement of Body weight changes after KKC treatment***

Drug treatment	Periodical Weight changes after KKC treatment					
	Day0	Day1	Day2	Day4	Day8	Day14
Normal control	233.33±3.575	234.5±2.513	239.33±2.813	243.16±2.857	245.66±2.14	252.33±1.82
Diabetic control	211.83±2.949	217±1.915	231.66±2.201	192.16±3.167	194.33±2.348	184.5±4.978
KKC 400mg/kg	212.33±1.406**	228±1.668^{ns}	214.5±1.40**	218±2.082**	233±1.759*	229±2.136**
Glibenclamide (0.5mg/kg)	216±3.46**	226±1.03*	214.16±2.61**	228.33±1.20**	229.83±2.22**	242±3.25^{ns}
Zinc Insulin (4.U/kg)	219.33±3.85*	232±1.83^{ns}	222.33±1.49**	219±1.77**	237±4.88^{ns}	238±4.123*

n = 6; Values are expressed as mean ± S.E.M.; ^{a}P<0.05 VS Normal control

Table: 3 ***Fasting serum Glucose concentration is normal and Alloxan-induced diabetic rats after KKC treatment***

Treatment	Fasting serum Glucose concentration (mg/dl) measured at regular intervals			
	I	IV	VIII	XIV
Normal Group	72±1.155	72.83±1.249	68.33±1.667	73.5±0.885
Diabetic Control	398.33±5.2	410.33±10.256	424.33±17.862	395.66±5.251
KKC (400mg/kg)	97±6.126**^{b}	382±8.66**^{a}	390±10.280^{a}	382±4.22^{a}
Glibenclamide/ Metformin (0.5:40mg/kg)	75.33±1.764^{b}	94.66±1.764^{b}	95.66±1.585^{b}	106±1.932^{b}
Zinc Insulin (4 U)	72±1.46^{b}	70±1.26^{b}	65.16±1.60^{b}	68.33±1.057^{b}

n=6; Values are expressed as mean ±S.E.M *P <0.05 Vs Normal, ^{a}P <0.05; ^{b}P <0.01 Vs Diabetic Control

Table: 4 ***Total Cholesterol and Triglyceride levels in normal and Alloxan-induced diabetic rats after KKC treatment***

Treatment	Dose	Parameters (mg/dl)	
		Total Cholesterol	Triglycerides
Normal control	10mg/kg of vehicle	83±1.528	93.33±1.606
Diabetic control	-	126.16±1.641	129.16±1.97
KKC	(400mg/kg)	74.83±1.47**^{b}	72.5±1.54**^{b}
Glibenclamide/Metformin	(0.5:40mg/kg)	84±1.46^{b}	86.66±1.76*^{b}
Zinc Insulin	(4 U/ml)	73.5±1.89**^{b}	67.33±1.52**^{b}

n=6; Values are expressed as mean ±S.E.M *P <0.05 Vs Normal, ^{a}P <0.05; ^{b}P <0.01 Vs Diabetic Control

Rat was used since it was routinely used animal model for quick screening of drugs for their hypoglycaemic/antihyperglycaemic 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. According to the standard working protocol, 14 days daily treatment with test drug moderately reduced the elevated blood glucose in alloxan induced diabetic rats while it had no effect on blood glucose of normal rats. The antidiabetic activity of *Kovai Kizhangu 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 280-340 mg/dL in different rats after stabilization period of 15days. Single dose (400 mg/kg body weight, oral) treatment with the drug produced 33% antihyperglycaemic effect (antidiabetic effect). Finally it can be concluded that KKC was found to possess moderate ($P<0.05$) antidiabetic action in alloxan induced diabetic rats.

CONCLUSION

According to the standard working protocol, 14 days daily treatment with test drug *Kovai Kizhangu 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 *Kovai Kizhangu Chooranam* may be attributed to the active ingredients present in the drug. Finally it can be concluded that *Kovai Kizhangu Chooranam* was found to possess remarkable ($P<0.01$) anti-diabetic action in alloxan induced diabetic rats.

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