

# Evaluation of Anti-Hyperglycemic Activity of Kariveppilai Churnam in Alloxan Induced Diabetic Rats

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## ABSTRACT

**Objective** - The present investigation is undertaken to study the effect of Kariveppilai churnam on changes in Body weight, Plasma glucose, Plasma Insulin, Hemoglobin and glycosylated hemoglobin and lipid profile in alloxan induced Diabetic Rats.

**Methodology** - The raw materials of the compound herbal drug were purchased, identified and pulverized into powder form. The trial drug was administered to alloxan induced diabetic male albino rats. The rats were divided into 5 groups with 6 rats in each group. Group 1, 2 and 3 were normal control, toxic control and diabetic control respectively. Group 4 and 5 received the trial drug at a dose of 250 mg/kg and 500 mg/kg orally respectively once a day. The anti-hyperglycemic activity of the trial drug was evaluated using the parameters of blood glucose, hemoglobin, glycosylated hemoglobin, plasma insulin, total cholesterol, triglycerides, HDL and phospholipids.

**Results** – The administration of the trial drug at doses of 250 mg/kg and 500 mg/kg of the body weight of the diabetes induced rats showed significant decrease in the above mentioned elevated parameters. Glipizide (10mg/kg) was used as the reference standard.

**Conclusion**- From the results it can be observed that trial drug at a dose of 250 mg/kg and 500 mg/kg has protective effect against alloxan induced diabetes and its complications. The findings suggest that kariveppilai churnam possess anti-atherogenic property along with anti hyperglycemic activity.

**KEYWORDS** – Diabetic complications, Alloxan, Kariveppilai churnam, Siddha

## INTRODUCTION

Siddha system of medicine is an indigenous system wherein all the creation and genesis of matter on earth are controlled and regulated by the pancha bhootas – the five elements, tridoshas and dasa naadi at microcosm and macrocosm plane. An imbalance in the creative forces subsequently causes defective function affecting the existence, qualitatively and quantitatively. Neerizhivu or mega neer is a condition where pathologically there is excessive urination. Twenty different types of mega neer have been elaborated in literatures by multitudinal time-honoured sages of immense spiritual insight. Yugi muni enunciates about neerizhivin avathaigal, complications consequential of long term uncontrolled neerizhivu.[1]

Diabetes mellitus is a metabolic disorder in which the body does not produce or properly utilize insulin. It causes disturbance in carbohydrate, protein and lipid metabolism leading to complications such as retinopathy, micro-angiopathy, nephropathy and atherosclerosis. In practical terms, diabetes mellitus is a condition where a profound alteration in the concentration and composition of lipid occurs.

In many diabetics the disease may be first detected when the patient presents with a complication. The patients also develop resistance to oral anti-hyperglycemic drugs when regularly consumed for longer periods.[2]

In such a scenario, an effective anti-diabetic drug which would exert a strict glycemic control and also prevent the further threat of the macro and micro-vascular complications is very much needed. The author intends to mend the broken ends in the treatment regimen of neerizhivin avathaigal with the trial drug kariveppilai churnam a multi herbal Siddha formulation.[3]

Despite the immense strides that have been made in the understanding and management of diabetes, the disease related complications are increasing unabated. In spite of the presence of known anti-diabetic medicine in the pharmaceutical market, remedies from medicinal plant are used with success to treat this disease. Many traditional plants treatments for diabetes are used throughout the world and there is an increasing demand by patients to use the natural products with anti-diabetic activity.

## MATERIALS AND METHODS

Materials:

Animals :

Male albino wistar rats (180-220gm).

Drugs :

The raw materials required for the preparation of Kariveppilai churnam were purchased from the local market, Tirunelveli Town and its botanical identity were confirmed by the Department of Gunapadam, Government Siddha Medical College, Palayamkottai. The trial drug was prepared as per the method described in the Siddha literature- Sarabendrar vaithiya rathnavalli.

Chemical:

Alloxan monohydrate (S. D Fine. Chem. Ltd, Mumbai).

Selection & acclimatization of animals:

Wistar strains of male albino rats weighing between 180-220gm are used for this study. The animals were housed in large spacious cages and they were fed with commercial pellets and access to water ad libitum. The animals were well acclimatized to the standard environmental condition of temperature ( $22^{\circ}\text{C} \pm 50^{\circ}\text{C}$ ) and humidity ( $55 \pm 5\%$ ) and 12 hr light dark cycles throughout the experimental period.

Induction of diabetes mellitus

Diabetes mellitus is induced in wistar rats by single intra peritoneal injection of freshly prepared solution of Alloxan monohydrate (150mg/kg BW) in physiological saline after overnight fasting for 12hrs.[4]

Alloxan is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the  $\beta$ -cells of pancreas possibly by generating the excess reactive oxygen species such as  $\text{H}_2\text{O}_2$ ,  $\text{O}_2$  and  $\text{HO}^-$ . The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrs post Alloxan injection. The rats with fasting plasma glucose level of 160-220mg/dl were used for this experiment. The study of the trial drug was conducted in KM college of pharmacy, Madurai, the experiment being approved by IAEC/KMCP/136, 2014.

Experimental procedure:

In the experiment a total of 30 rats (24 diabetic surviving rats & 6 normal rats) were used. Diabetes was induced in rats 3 days before starting the experiment. The rats were divided into 5 groups after the induction of Alloxan diabetes. In the experiment 6 rats were used in each group.

TREATMENT PROTOCOL

Group 1: (Normal control) consist of normal rats given with 10ml/Kg of normal saline, orally.

Group 2: (Toxic control) Diabetic control received 150mg/Kg of Alloxan monohydrate intra peritoneally.

Group 3: Diabetic control received glipizide at a dose of (10mg/Kg orally) for 28 days.

Group 4: Served as a treatment control group and was administered with kariveppilai churnam at a dose of 250mg/kg orally.

Group 5: Served as a treatment control group and was administered with kariveppilai churnam at a dose of 500mg/kg orally.

## METHODOLOGY

Sample collection:

After 28 days of treatment, body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids were determined. Blood was collected from the eyes (venous pool) by sino-ocularpuncture [5] in EDTA coating plasma tubes for the estimation of blood parameters.

## BIOCHEMICAL ANALYSIS

Estimation of blood glucose:

Blood glucose was estimated by commercially available glucose kit (One Touch Ultra) Johnson Johnson based on glucose oxidase method. [6]

Plasma insulin:

Plasma insulin was determined by ELISA method using a Boehringer – Mannheim kit[7] with an ES300 Boehringer analyzer (Mannheim, Germany).

Estimation of total haemoglobin and glycosylated haemoglobin:

Total haemoglobin was determined by the method of Drabkin and Austin (1932) [8] and glycosylated haemoglobin was determined by the method of Sudhakar Nayak and Pattabiraman (1981). [9]

Estimation of lipid & lipoprotein:

Plasma lipids were determined by auto analyzer according to the method of Parkeh and Jung (1970) (total cholesterol), [10] Gidez and Webb (1950) (HDL-cholesterol), [11] Zilversmith and Davis (1950) (phospholipids) [12] and Rice (1970) (triglycerides). [13]

Statistical analysis:

The data for various biochemical parameters were analyzed using analysis of variance (ANOVA), and the group means were compared by Newman-Keuls multiple range test (NKMRT). Values were considered statistically significant at  $p < 0.01$ .

## RESULTS

Table no: 1 illustrates the levels of initial and final blood glucose, and change in body weight, in normal rats, and treatment control animals in each group. The mean body weight of diabetic rats (G2) was significantly decreased as compared to normal control rats. The body weight of diabetic control rats treated with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg increased the body weight non-significantly as compared to normal control animals.

Fasting blood glucose level was significantly increased  $220.40 \pm 6.60$  in diabetic animals as compared to normal animals. However the level of fasting blood glucose, returned to near normal range in diabetic rats treated with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg.

Table no: 2 illustrates the levels of total hemoglobin, glycosylated hemoglobin and plasma insulin in normal rats and treatment control animals in each group.

The levels of total hemoglobin, and plasma insulin levels were decreased significantly where as glycosylated hemoglobin levels were increased significantly as compared to normal control rats. However the level of total hemoglobin, glycosylated hemoglobin and plasma insulin, returned to near normal range in diabetic rats treated with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg

Table no: 3 shows the level of serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), Low density lipoprotein (LDL) and phospholipids of normal and experimental animals in each group.

Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, where as HDL cholesterol level was decreased in Alloxan induced diabetic rats as compared to normal rats. Treatment of normal and Alloxan induced diabetic rats with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg for 28 days resulted in marked decrease in total cholesterol, triglycerides, Low density lipoprotein (LDL) and phospholipids levels and increase in HDL-C levels as compared to Alloxan induced diabetic rats.

## DISCUSSION

Alloxan causes massive reduction in insulin release, through the destruction of  $\beta$ -cells of the islets of Langerhans. The mechanism of Alloxan action was fully described elsewhere (Lazarow, 1964; Colca et al., 1983). [14,15] In our study, we have observed a significant increase in the plasma insulin level when Alloxan induced diabetic rats were treated with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg, this could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing  $\beta$ - cells of islets of Langerhans or its release from bound insulin.

In uncontrolled or poorly controlled diabetes there is an increased glycosylation of a number of proteins including haemoglobin and  $\alpha$ -crystalline of lens (Alberti and Press, 1982). [16] Glycosylated haemoglobin (HbA1C) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) [17] and the amount of increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979). [18] During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in Alloxan induced diabetic rats (Sheela and Augusti, 1992). [19] Administration of kariveppilai churnam at a dose of 250mg/kg and 500mg/kg for 28 days prevents a significant elevation in glycosylated haemoglobin there by increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycemic control produced by kariveppilai churnam at a dose of 250mg/kg and 500mg/kg.

The body weight was decreased in Alloxan induced diabetic rats. Administration of kariveppilai churnam at a dose of 250mg/kg and 500mg/kg increased the body weight in Alloxan induced diabetic rats. The ability of kariveppilai churnam at a dose of 250mg/kg and 500mg/kg to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia.

The level of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents the risk of coronary heart disease (CHD). [20] Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk of vascular disease. [21] The abnormal high concentration of serum lipids in diabetic subjects is mainly due to increased mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase. However, glucagon, catecholamines and other hormones enhance lipolysis. The marked hyperlipidaemia that characterized the diabetic state may therefore be regarded as a consequence of the uninhibited actions of lipolytic hormones on the fat depots.

In the Alloxan induced diabetes mellitus, the rise in blood glucose is accompanied by an increase in serum cholesterol and triglycerides. The levels of cholesterol and triglycerides and Low density lipoprotein(LDL) levels were brought to near normal by the treatment with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg in Alloxan induced diabetic rats.

The effect of kariveppilai churnam at a dose of 250mg/kg and 500mg/kg on diabetic hypertriglyceridemia could be through its control of hyperglycemia. This is in agreement with the facts that:

1. The level of glycemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride concentrations.[22]
2. Improved glycemic control following sulfonylurea therapy decreases the levels of serum VLDL and total triglycerides. [23]

The main 'anti-atherogenic' lipoprotein (HDL) is involved in the transport of cholesterol from peripheral tissues into liver [24] and thereby it acts as a protective factor against coronary heart disease (CHD). [25]

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats. [26] Our results clearly show that the level of HDL-cholesterol was increased in Alloxan induced diabetic rats when treated with kariveppilai churnam at a dose of 250mg/kg and 500mg/kg. These results suggest that kariveppilai churnam at a dose of 250mg/kg and 500mg/kg has protective effect against Alloxan-induced diabetes and its complications.

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**Table No: 1**

Effect of KARIVEPPILAI CHURNAM on initial and final body weight and blood glucose in normal and treated animals.

GROUP	Body weight (g)		Blood glucose (mg / 100ml) Initial	Blood glucose (mg / 100ml) Final
	Initial	Final		
G1	218 ± 7.30	232 ± 8.20	85.65 ± 3.30	88.70 ± 3.56
G2	215 ± 6.90	180 ± 4.70** <sup>(a)</sup>	85.75 ± 3.85	220.40 ± 6.60** <sup>(a)</sup>
G3	235 ± 7.60	220 ± 7.30	87.50 ± 4.20	112.30 ± 4.55** <sup>(b)</sup>
G4	220 ± 7.28	235 ± 8.15	83.78 ± 3.58	132.45 ± 5.20** <sup>(b)</sup>
G5	235 ± 7.24	220 ± 7.40	86.50 ± 3.75	125.40 ± 4.45** <sup>(b)</sup>

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- \*\* (a) Values are significantly different from normal control G1 at P<0.001.
- \*\* (b) Values are significantly different from Diabetic control G2 at P<0.01.

**Table No:2**

Effect of KARIVEPPILAI CHURNAM on plasma insulin, Hemoglobin & Glycosylated hemoglobin in normal and treated animals.

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA1 (%)	Plasma Insulin (µU/ml)
G1	12.90 ± 1.55	0.35 ± 0.06	35.50 ± 2.90
G2	6.10 ± 0.72** <sup>(a)</sup>	0.99 ± 0.14** <sup>(a)</sup>	13.70 ± 1.70** <sup>(a)</sup>
G3	12.15 ± 1.20** <sup>(b)</sup>	0.40 ± 0.07** <sup>(b)</sup>	29.55 ± 2.50** <sup>(b)</sup>
G4	11.30 ± 0.95** <sup>(b)</sup>	0.49 ± 0.09** <sup>(b)</sup>	24.70 ± 2.30** <sup>(b)</sup>
G5	11.90 ± 1.12** <sup>(b)</sup>	0.44 ± 0.05** <sup>(b)</sup>	27.55 ± 2.50** <sup>(b)</sup>

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- \*\* (a) Values are significantly different from normal control G1 at P<0.001.
- \*\* (b) Values are significantly different from Diabetic control G2 at P<0.01.

**Table No.3**

Serum lipids of Normal and experimental groups.

<b>GROUPS</b>	<b>Total Cholesterol  (mg/dl)</b>	<b>Triglyceride  (mg/dl)</b>	<b>HDL-C  (mg/dl)</b>	<b>Phospholipids  (mg/dl)</b>	<b>LDL  (mg/dl)</b>
<b>G1</b>	84.90 ± 2.55	88.55 ± 2.50	50.32 ± 1.80	122.70 ± 2.42	15.40 ± 1.52
<b>G2</b>	220.28± 6.75** <sup>(a)</sup>	150.65 ± 4.60** <sup>(a)</sup>	32.40 ± 1.18** <sup>(a)</sup>	210.30 ± 6.28** <sup>(a)</sup>	38.95± 2.35** <sup>(a)</sup>
<b>G3</b>	110.90 ± 3.30** <sup>(b)</sup>	93.80± 2.55** <sup>(b)</sup>	44.95 ± 1.45	140.50 ± 3.95	20.30± 1.90** <sup>(b)</sup>
<b>G4</b>	125.65 ± 3.60** <sup>(b)</sup>	110.80 ± 2.95** <sup>(b)</sup>	40.50 ± 1.42** <sup>(b)</sup>	158.55 ± 4.08** <sup>(b)</sup>	29.55± 1.95** <sup>(b)</sup>
<b>G5</b>	118.50 ± 3.45** <sup>(b)</sup>	99.30 ± 2.72** <sup>(b)</sup>	42.52 ± 1.70** <sup>(b)</sup>	150.32± 3.95** <sup>(b)</sup>	24.25± 1.80** <sup>(b)</sup>

- Values are expressed as mean ± SEM.
- Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests.
- \*\* (a) Values are significantly different from normal control G1 at P<0.001.
- \*\* (b) Values are significantly different from Diabetic control G2 at P<0.01.