Original Research Paper

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THE ANTI HYPERLIDEMIC AND HYPOGLYCEMIC EFFECT OF SIDDHA FORMULATION KARUVEPILLAI CHOORANAM IN EXPERIMENTAL DIABETES AND THEIR EFFECTS ON METABOLIC ENZYMES INVOLVED IN CARBOHYDRATE METABOLISM

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder which is financial burden on national health care systems. During this condition body does not produce or properly utilize insulin and leading to disturbance in carbohydrates, proteins and lipids metabolisms and in later leads to complications such as CAD, Retinopathy, Neuropathy and Nephropathy. In 2014, the IDF estimated that 8.2% of aged 20–79 (387 million people) were living with diabetes and obesity. In 2018 2-2.5 million people are affected anobesity, out of which a whooping 40-60% have diabetesmelitus (Diabesity),nearly 80% of indian type2 diabetic patients are nonobese,where as 60-80% of such diabetics in the West are Obese (Das 1994,Sahay BK 1993).

Objective: The present study is to investigate ,the effective role of Karuvepillaichooranam on changes in Body weight, Plasma glucose, Haemoglobin ,glycosylated haemoglobin and lipid profile.

Materials and methods: Streptozotocin induced Wistermale albino rats weighing between 180-220gms are used in this study. The rats were divided into 5 groupseach grouphave6 rats, totaly30 albino rats used. The Karuvepillaichooranam (KVC) 100 and 200 mg/kg dividing dosewere administered orally for 28 days. The end of study showed , body weight, blood glucose, haemoglobin, glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids and glycogen content and antioxidant enzymes level were determined.

Result: The significant increase in the plasma insulin level and reduced glycosylated haemoglobin, thereby kvc is reduced hyperglycaemia and body weight, increasing the level of total haemoglobin in diabetic rats and protect massive body weight loss seems to be due to its ability to reduce hyperglycaemia. The levels of Lipid profiles are decreased and HDL-cholesterol was increased. Significantly increased in hexokinase, Glucokinase activity and glucose-6-phosphate level.

KEYWORDS : Anti-hyperglycaemic effect,Metabolic enzymes, Carbohydrate metabolism.

INTRODUCTION

Diabetes mellitus is a metabolic disorder, which isfinancial burden on national health care systems. During this condition body does not produce or properly utilize insulin and leading to disturbance in carbohydrate, protein and lipid metabolism. In later the diabetes, produce many multifocal lesions such as retinopathy, neuropathy and nephropathy.

The diabetes is growing up rapidly in low and middle-income countries andmiddle age groups. In 2035, Indiawill raised from 66.8 to 120.9 million people, the IDF estimated that 8.2% of adults aged 20–79 were living with diabetes (Joao da Rocha Fernandez et al 2014). In India is a second largest number in diabetes cases(73 million in 2018), an estimated 46% of cases currently undiagnosed (IDF, 2018),and poor control diabetes can increasedrisk of diabetes-related complications. An estimated 47% of people with diabetes were aged between 40and 59, with 77% living in low- and middle-income countries.Diabetes was responsible for the deaths of an estimated 4.9 million people worldwide in 2014

Despite the immense strides that have been made in the understanding and management of diabetes the disease and 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.So, KVC is a highly potential to control diabetes and dyslipidaemia.

MATERIALS AND METHODS:

The eight individual ingredients was collected from south zone of tamilnadu, india.Which it was identified by Medicinal plant experts and siddha pharmacologist at government siddha medical college, palayamkottai,Tirunelveli. Equal ratio in sl.no1 to7and sl.no 8 is seven per centincrease in total weight of 1to 7.The eight ingredients are

Table no1. Ingridents Ofkariveppillai Choornam

Sl.no	INGRITENTS	IMPORTANT ALKALOIDS
1	MurrayaKoenjii(L) Sperng	Mahanimbine,Koenimbine,Koeng icine
2	Gossypiumherbaceum (L)	Gossypin,Quercetin-3-0-glucoside
3	Curcuma longa (L)	Curcumin,Demethoxycurcumin
4	Cosciniumfenestratum (Gaertn.)Colebr	Berlambine,Berberine,Sitosterol, Stigmasterol
5	Terminaliachebula (Retz)	Chebulin,Phenoliccompounds,Gal lic acid
6	Terminaliabellerica (Gaertn.)Roxib	Gallic acid, Ellagicacid, Chebulagicacid, Cardio glycoside
7	Emblicaofficinalis (Gaertn)	Terchebin,Ellagicacid,Phyllembic acid
8	Salaciareticulata (wall.ex)	Leucopelargonidin,Friedelan

IN VITRO-EXPERIMENTAL MODEL

INDUCTION OF DIABETES MELLITUS

A Streptozotocin (S. D Fine. Chem. Ltd, Mumbai) induced Male albino wistar rats (180-220gm) were used this study, a single intraperitoneal injection of freshly prepared solution of Streptozotocin (25mg/kg BW) in physiological saline after overnight fasting for 12hrs (Ai-Shamaony et al.1994). The development of hyperglycaemias in rats is confirmed by plasma glucose estimation 72 hrs.post Streptozotocin injection. The rats with fasting plasma glucose level of >180-220mg/dl were used for this experiment.

Experimental procedure:

In the experiment,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 Streptozotocin diabetes. In the experiment,6 rats were used in each group. Detailed given below

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

Group-II: (Toxic control) Diabetic control received 25mg/Kg of Streptozotocin through I.P.

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

Group-IV: Diabetic control received Karuvepillai Chooranam at a dose of (100mg/Kg orally) for 28 days.

Group-V: Diabetic control received Karuvepillai Chooranam at a dose of (200mg/Kg orally) for 28 days.

After 28 days of treatment, body weight, blood glucose, haemoglobin,

glycosylated haemoglobin, plasma insulin, total cholesterol, triglycerides, HDL-cholesterol and phospholipids and glycogen content and antioxidant enzymes level were determined. The estimation of blood glucoselevel was evaluated by Johnson Johnson based on glucose oxidase method (Way forth 1980,Trinder,1969). Plasma insulin Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kit (Anderson et al.1993) with an ES300 Boehringer analyser (Mannheim, Germany).Total haemoglobin was determined by the method of Drabkin and glycosylated haemoglobin was determined by the method of (Drabkin and Austin,1932) and Plasma lipids were determined by auto analyser according to the method of Parkeh and Jung (1970) (total cholesterol), (Parkeh andJung,1970 Gidez and Webb 1950) , The HDL-cholesterol ,TGL and phospholipids are use to determined [Rice 1970).Hepatic glucokinase and hexokinase activity was carried out spectrophotometric methodology (Crane et al. 1955). The determination of phosphoric acid concentration in assay mixture was done calorimetrically (12) (Fiske et al., 1925).Glycogen content was determined calorimetrically.(13) (Morales et al., 1973).

DISSCUSSION AND RESULTS:

Table No1 a.Effect of Karuvepillaichooranam on initial and final body weight and blood glucose in normal and treated animals.

Group	Body weight (g) Initial	Body weight (g) Final	Blood glucose (mg / 100ml)Initial	Blood glucose (mg / 100ml) Final
G1	235 ± 6.05	242 ± 6.10	86.60 ± 3.45	91.75 ± 3.18
G2	225 ± 5.54	170 ± 7.25**(a)	88.30 ± 3.70	214.46 ± 5.82**(a)
G3	232 ± 7.44	238 ± 7.30	90.64 ± 4.25	124.45 ± 4.35**(b)
G4	230 ± 7.20	240 ± 7.35	88.75 ± 3.65	146.45 ± 7.26**(b)
G5	230 ± 7.35	238 ± 7.35	90.42 ± 3.62	155.48 ± 4.62**(b)

According to **Table no 1(a).**Values were considered statistically significant at p<0.01. ** (a) Values are significantly different from normal control G1 at P<0.001 and** (b) Values are significantly different from Diabetic control G2 at P<0.01.The body weight was decreased in Streptozocin diabetic rats. Karuvepillaichooranam at a dose of 100mg/kg and 200mg/kg increases the body weight in Streptozocin induced diabetic rats.

Table no: 2-Effect of Karuvepillaichooranam on plasma insulin, HbA1C level:

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA1C (%)	Plasma Insulin (µU/ml)
G1	13.80 ± 1.52	0.40 ±0.06	33.48 ± 2.75
G2	6.65 ± 0.52**(a)	0.90 ±0.14**(a)	13.58 ± 1.60**(a)
G3	14.30 ± 1.30**(b)	0.42 ±0.07**(b)	32.35 ± 2.42**(b)
G4	12.54 ± 0.92**(b)	0.50 ±0.08**(b)	30.80 ±2.60**(b)
G5	12.64 ± 1.22**(b)	0.45 0.04**(b)	29.70 ±2.60**(b)

** (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&3. illustrates ,the levels of total haemoglobin, and plasma insulin levels and HbA1C were decreased significantly where as glycosylated haemoglobin levels were increased significantly as compared to normal control rats.

Table No.3-Serum lipids of Normal and experimental groups.

GROUPS	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)
G1	88.80 ±2.68	92.48 ±2.65	56.50 ±1.85	125.65 ±2.43	18.35 ± 1.40
G2	228.48 ± 7.52**(a)	160.65 ± 4.65**(a)	35.60 ± 1.34**(a)	220.48 ± 6.30**(a)	42.65±2.50**(a)
G3	118.90 ± 3.36**(b)	98.90 ± 2.46**(b)	45.20 ± 1.45	154.45 ± 3.95	27.80±1.80**(b)
G4	130.60 ± 3.58**(b)	120.86 ± 2.90**(b)	43.25 ± 1.38**(b)	162.60 ± 4.15**(b)	33.28±1.55**(b)
G5	123.40 ± 3.35**(b)	104.65 ± 2.80**(b)	40.25 ± 1.58**(b)	153.40 ± 3.82**(b)	28.35±1.76**(b)

According to **Table 2 &3** showed ** (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. To compared standard group III, Group IV and V was significantly reduced in Lipids and Plasma insulin, HbA1c level.

Table 4 Effect of Karuvepillaichooranam on glycogen content (mg/gm tissue)

Groups	Liver Tissue Glycogen Content (mg/g tissue)
G1	44.30 ± 3.50
G2	12.28 ± 0.82*a
G3	36.50 ± 1.74*b
G4	28.40 ± 1.35*b
G5	30.59 1.50*b

According to Table 4 and 5 showed, Group IV and V liver tissue

glycogen contend was markedly reduced in compared group III. (a) and (b) Values are significantly different from normal control G1 at P<0.001 and other liver enzymes like hexokinase, Glucose -6-phosphate and glucokinase was significantly different from normal control G1 at P<0.001.it was different from Diabetic control G2 at P<0.01Glycogen content of liver tissue .was estimated on the 28th day in non-diabetic control, diabetic control drug, treated group and positive control group.

Table 5 :Effect of Karuvepillaichooranam on enzymes involved in carbohydrate metabolism in rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
G1	0.215 ± 0.014	0.395 ± 0.012	27.38± 1.40
G2	0.093 ± 0.005*a	0.130 ± 0.008*a	6.62 ± 0.30*a
G3	0.131 ± 0.008*b	0.306 ± 0.012*b	19.22 ± 0.90*b
G4	0.125 ± 0.006*b	0.234 ± 0.006*b	16.10 ± 0.45*b
G5	0.144 ± 0.007*b	0.245 ± 0.007*b	16.30 ± 0.52*b

Table no 6.The respective percentage decrease was 56.19%, 79.96% and 67.69% in diabetic control. Treatment with Karuvepillaichooranam at a dose of 100mg/kg and 200mg/kg for 28 days led to rise in percentage of these parameter by 22.03%, 56.03%, and 45.21% , 47.5%, 33.33% and 67.88% respectively (P<0.001) as compared to diabetic control. Also, treatment with Glipizide 10mg/kg for 28 days led to rise in percentage of these parameters by 27.55%, 65.39% and 58.0% respectively (P< 0.001) as compared to diabetic control. In liver homogenate, there was significant decrease in SOD, CAT and GPx levels and increase in LPO levels were observed in animals treated with streptozocin 25mg/kg (group II) as compared to normal control group (Group I).

Table.No:6Effect of Karuvepillaichooranam treatment on biochemical parameter in streptozotocin induced toxicity.

Group. No.	SOD (U/mg) Protein	CATALASE (U/mg) Protein	GPX (U/mg) Protein	MOA (U/mg) Protein
G1	135.30±2.40	295.45±2.40	1.22±0.08	3.96±0.18
G2	*a 73.26±1.43	*a 195.80±2.75	*a 0.45±0.03	*a 7.48±0.15
G3	*b 123.10±2.82	*b 265.45±1.90	*b 0.91±0.05	*b 5.56±0.15
G4	*b 97.50±1.65	*b 235.10±1.75	*b 0.70±0.04	*b 5.65±0.30
G5	*b 110.68±2.65	*b 245.80±2.70	*b 0.75±0.05	*b 4.90±0.07

CONCLUSION:

The level of glycaemic control is the major determinant of total and very low density lipoprotein (VLDL), triglyceride, concentrations improvedglycaemic control following sulfonylurea therapy, decreased serum VLDL, TGL levels. And total triglycerides (Laakso,1995). HDL-cholesterol was increased in Streptozocin induced diabetic rats when treated with Karuvepillaichooranam at a dose of 100mg/kg and 200mg/kg. These results suggest that Karuvepillaichooranam at a dose of 100mg/kg and 200mg/kg has protective effect against Streptozocin induced diabetes and its complications.Decreased enzymatic activity of Hexokinase, Glucokinase and substrate glucose-6- phosphate has been reported in diabetic animals resulting in depletion of liver and muscle glycogen.(Hikino et al.1989).

So, Kariveppillaichoornam is Significantly exhibited inantioxida seenzymes, blood sugar and lipid levels.

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