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ANTI-HYPERGLYCEMIC EFFECT AND EFFECT OF ANTI -OXIDANT ENZYMES INVOLVED IN METABOLISM OF *SEENDHIL SARKKARAI* IN EXPERIMENTAL ANIMAL

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ABSTRACT

Background: *Seendhil* (*Tinospora cordifolia)* is widely used for medicinal purpose. *Seendhilsarkkarai*has been used traditionally as anti-hyperglycemic in Siddha System of medicine.**Objectives:** The present study was undertaken to evaluate the anti–hyperglycemic and anti-hyperlipidemic effects on anti – oxidant enzymes involved in metabolism of *seendhilsarkkarai*in Streptozotocin induced diabetic rats. **Materials and method:** Wistar strains of male albino rats weighing between 180-220gm are used for this study. Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of Streptozotocin (25mg/kg BW) in physiological saline after overnight fasting for 12hrs**.**The body weights of the rats in every group were recorded weekly. five groups of 6 animals in each received normal saline for normal control, glipizide at a dose of (10mg/Kg orally) for diabetic control, *SeendhilSarkkarai* at a dose of (100mg/Kg orally) and 200mg/kg for euglycemic rat 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. Blood was collected from the eyes (venous pool) by sino-ocularpuncture. The data was analyzed statistically using analysis of variance (ANOVA), and the group means were compared by Newman-Keul's multiple range test (NKMRT) **Results:** Total cholesterol, triglycerides, high density lipoprotein, Low density lipoprotein (LDL) and phospholipids levels were significantly increased, whereas HDL-C level was decreased in streptozotocin induced diabetic rats as compared to normal rats. Treatment of normal and streptozotocin induced diabetic rats with ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg (*SEENDHIL SARKKARAI)* 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 streptozotocin induced diabetic ratsrespectively ($P<0.001$) as compared to diabetic control.**Conclusion:** Present study revealed that the *seendhilsarkkarai*has anti hyperglycemic and anti-hyperlipidemicaction and can be safely used in the treatment of mild to moderate cases of hyperlipidemia.

KEY WORDS

Tinospora cordifolia, seendhil sarkkarai, streptozotocin, Diabetic Rats, anti–hyperglycemia, anti-hyperlipidemia, carbohydrate metabolism

INTRODUCTION

Diabetes mellitus is a carbohydrate metabolism disorder of endocrine system due to absolute or relative deficiency of insulin secretion, action, or both. It causes disturbance in carbohydrate, protein and lipid metabolism and complications such as retinopathy, microangiopathy and nephropathy. In practical terms, diabetes mellitus is a condition in diabetes, a profound alteration in the concentration and composition of lipid occurs. The global figure of people with diabetes set rise from the current estimate of 150-220 million in 2010 and 300 million in 2025.

The NIIDM, type 2 is the most prevalent form globally which is associated with elevated postprandial hyperglycemia. Pancreatic enzymes in the digestive system and catalyses the initial step in hydrolysis of starch to maltose finally to glucose. Degradation of this dietary starch proceeds rapidly and leads to elevated postprandial hyperglycaemia. It has been shown that activity of enzymes in the small intestine correlates to an increase in postprandial glucose levels, the control of an important aspect in treatment of diabetes. Hence retardation of starch digestion by decreased enzymatic activity α-Amylase, Hexokinase,

Glucokinase and substrate glucose-6- phosphate resulting in depletion of liver and muscle glycogen would play a key role in the control of diabetes mellitus.

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.

Since Siddhar's period, many herbs have been in use for treating diabetes. It is found that diabetes mellitus is invariably associated with carbohydrate, protein and lipid metabolism. So it becomes mandatory to take care of lipid level in diabetic patients along with blood sugar levels as it is known to increase the morbidity and mortality if neglected. Therefore there is need for drug with both antidiabetic and hypo-lipidemic activity. In this regard, Seendhil sarkkarai plays crucial role.

SEENDHIL SARKKARAI

Tinisporacordifolia

The isoquinoline alkaloid rich fraction from stem, including, palmatine, jatrorrhizine, and magnoflorine have been reported for insulin-mimicking and insulin-releasing effect both in vitro and in vivo.

The present investigation is undertaken to the study the effect of *SeendhilSarkkarai*on changes in Body weight, Plasma glucose, Hemoglobin and glycosylated hemoglobin and lipid profile, metabolic enzymes and antioxidant enzymes levels.

MATERIALS AND METHODS

The present study was carried out at the K.M College of pharmacy, Madurai after obtaining the permission from the Institutional animal ethical committee. (IAEC No: TNMGRMU/KMCP/IAEC/312 /2017).

STANDARD OPERATIVE PROCEDURE OF *SEENDHILSARKKARAI*

All the ingredient of herbal formulation was purified according to the suitable procedure methods described in Siddha classical literature.

Cut and removed the outer covering of aged stem of *Tinosporacordifolia* was shade dried and made into powder. Added 1400 ml water and kneaded well, then mixed 5600ml water and allowed to precipitating. The flour of *TinosporaCordifolia* was precipitated in the latter. After filtered water and again added 5600 ml water into it and allowed to precipitating. It was done for 10 times. Then added *kaadineer* mixed with lemon juice (16:1) were allowed to precipitating for one day. Like another day buttermilk and lemon juice (16:1) were allowed to precipitating. The ratio should be maintained to flour and solution is 1:4. Finally *Sendhil* flour was collected and dried.

MATERIALS

Animals :Male albino wistar rats (180-220gm)

Drugs : ***SeendhilSarkkarai***

Chemical:Streptozotocin (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 (22^0c ± 5^0c) and humidity (55 ±5%) and 12 hr light dark cycles throughout the experimental period.

INDUCTION OF DIABETES MELLITUS

Diabetes mellitus is induced in wistar rats by single intraperitoneal injection of freshly prepared solution of Streptozotocin (25mg/kg BW) in physiological saline after overnight fasting for 12hrs**.**

Streptozotocin is commonly used to produce diabetes mellitus in experimental animals due to its ability to destroy the β-cells of pancreas possibly by generating the excess reactive oxygen species such as H_20_2, 0_2 and $H0^-$. The development of hyperglycemias in rats is confirmed by plasma glucose estimation 72 hrspostsStreptozotocin injection. The rats with fasting plasma glucose level of >180-220mg/dl were used for this experiment.

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 Streptozotocin diabetes. In the experiment 6 rats were used in each group.

Treatment Protocol

- 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 *SeendhilSarkkarai* at a dose of (100mg/Kg orally) for 28 days.
- Group-V: Diabetic control received *SeendhilSarkkarai* at a dose of (200mg/Kg orally) for 28 days.

METHODOLOGY

Sample collection

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. Blood was collected from the eyes (venous pool) by sino-ocularpuncture.

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.

Plasma insulin

Plasma insulin was determined by ELISA method using a Boehringer–Mannheim kitwith 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)and glycosylated haemoglobin was determined by the method of SudhakarNayak and Pattabiraman (1981).

Estimation of lipid & lipoprotein

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

Hepatic glucokinase and hexokinase activity

The part of liver for each test was perfused with ice cold 0.15M KCl and 1mM EDTA solution and homogenized twice its weight of ice cold buffer (0.01 cysteine and 1mM EDTA in 0.1 ml Tris-HCL, pH 7.4) and centrifuged for 20 min at 4^0C. Glucose phosphorylation was assayed by means of glucose 6 phosphate dependent spectrophotometric method (Crane et al., 1955.

Glucose-6-phosphatase activity

The part of the liver for each test was homogenized with 40 times its weight of ice cold buffer (0.1 citrate-KOH, pH 6.5) and filtered through cheese cloth. Glucose-6-phosphatase activity was measured by phosphate release by the method Marjorie. The determination of phosphoric acid concentration in assay mixture was done calorimetrically(Fiske et al., 1925).

Glycogen Content

The tissue sample was digested by hot concentrated 30% KOH and treated with anthrone reagent. Glycogen content was determined calorimetrically. (Morales et al., 1973).

Statistical Analysis

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

RESULTS

Table No: 1

Effect of *Seendhil Sarkkarai*on Initial and Final Body Weight and Blood Glucose in Normal and Treated Animals

GROUP	Body weight (g)		Blood glucose (mg / 100ml)	Blood glucose (mg / 100ml)
	Initial	Final	Initial	Final
G1	243 ± 6.15	245 ± 6.17	86.65 ± 4.40	88.85 ± 3.22
G2	233 ± 5.61	176 ± 7.33**(a)	85.28 ± 3.72	215.35 ± 5.81**(a)
G3	237 ± 7.53	241 ± 7.35	87.68 ± 4.33	121.50 ± 4.32*(b)
G4	233 ± 7.29	245 ± 7.32	86.78 ± 3.68	144.38 ± 7.23**(b)
G5	239 ± 7.39	243 ± 7.42	90.46 ± 3.85	153.45 ± 4.66**(b)

- 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 *Seendhil Sarkkarai* on Plasma Insulin, Hemoglobin & Glycosylated Hemoglobin in Normal and Treated Animals

GROUPS	Haemoglobin (gm/100ml)	Glycosylated haemoglobin HbA1 (%)	Plasma Insulin (µU/ml)
G1	13.84 ± 1.62	0.45 ±0.07	37.24 ± 2.78
G2	6.50 ± 0.50**(a)	0.91 ±0.14**(a)	16.60 ± 1.63**(a)
G3	14.26 ± 1.47**(b)	0.46 ±0.06**(b)	35.35 ± 2.39**(b)
G4	12.41 ± 0.94**(b)	0.52 ±0.09**(b)	33.80 ±2.61**(b)
G5	12.60 ± 1.32**(b)	0.49 ±0.05**(b)	32.80 ± 2.63**(b)

- 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

GROUPS	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-C (mg/dl)	Phospholipids (mg/dl)	LDL (mg/dl)
G1	85.74 ±2.64	94.46 ±2.68	56.46 ±1.84	127.45 ±2.42	19.30 ± 1.40
G2	225.42±7.46**(a)	158.60±4.55**(a)	36.68 ±1.34**(a)	219.44±6.32**(a)	44.65±2.52**(a)
G3	114.86±3.34**(b)	100.90±2.42**(b)	48.18 ±1.44	155.40 ±3.92	28.74±1.76**(b)
G4	125.54±3.58**(b)	120.70±2.90**(b)	44.43±1.42**(b)	164.60±4.12**(b)	34.25±1.54**(b)
G5	120.43±3.40**(b)	105.40±2.74**(b)	43.47±1.59**(b)	155.40±3.82**(b)	30.34±1.72**(b)

- 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: 4

Effect of *Seendhil Sarkkarai* on Glycogen Content (Mg/Gm Tissue)

Groups	Liver Tissue Glycogen Content (mg/g tissue)
G1	46.30 ± 3.50
G2	14.24 ± 0.76*a
G3	38.50 ± 1.78*b
G4	30.42 ± 1.30*b
G5	32.64 ± 1.50*b

- 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: 5

Effect of *Seendhil Sarkkarai* on Enzymes Involved in Carbohydrate Metabolism in Rats

Groups	Hexokinase (µg/mg)	Glucose-6-Phosphate (µg/mg)	Glucokinase (µg/mg)
G1	0.216 ± 0.014	0.395 ± 0.010	29.42 ± 1.43
G2	0.096 ± 0.004*[a]	0.132 ± 0.007*[a]	8.58 ± 0.35*[a]
G3	0.132 ± 0.007*[b]	0.305 ± 0.010*[b]	21.22 ± 0.93*[b]
G4	0.127 ± 0.005*[b]	0.237 ± 0.007*[b]	18.15 ± 0.47*[b]
G5	0.147 ± 0.006*[b]	0.246 ± 0.008*[b]	18.35 ± 0.96*[b]

- 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: 6

Effect of Seendhil Sarkkarai 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	132.24±2.41	292.42±2.40	1.23±0.07	3.94±0.18
G2	*a69.23±1.44	*a191.88±2.73	*a0.47±0.02	*a7.46±0.16
G3	*b119.10±2.80	*b261.44±1.90	*b0.92±0.02	*b5.54±0.13
G4	*b95.52±1.55	*b231.10±1.75	*b0.76±0.02	*b5.67±0.26
G5	*b106.68±2.62	*b241.60±2.70	*b0.77±0.05	*b4.86±0.08

DISCUSSION

Diabetes mellitus is the commonest endocrine disorder and is as old as mankind. Since *Siddhar's* period, many herbs have been in use for treating diabetes. It is found that diabetes mellitus is invariably associated with carbohydrate, protein and lipid metabolism. So it becomes mandatory to take care of lipid level in diabetic patients along with blood sugar levels as it is known to increase the morbidity and mortality if neglected. Therefore there is need for drug with both antidiabetic and hypo-lipidemic activity. In this regard, *Seendhilsarkkarai* has efficacy of treating this condition.

Streptozocin causes massive reduction in insulin release, through the destruction of β-cells of the islets of Langerhans. In this study, it has observed a significant increase in the plasma insulin level when Streptozocin induced diabetic rats were treated with ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200 mg/kg this could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-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 α-crystalline of lens (Alberti and Press, 1982).Glycosylated haemoglobin (HbA_1C) was found to increase in patients with diabetes mellitus to approximately 16% (Koenig et al., 1976) and the amount of increase is directly proportional to the fasting blood glucose level (Jackson et al., 1979).During diabetes the excess glucose present in blood reacts with haemoglobin. Therefore, the total haemoglobin level is decreased in Streptozocin induced diabetic rats (Sheela and Augusti, 1992). Administration of ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg for 28 days prevents a significant elevation in glycosylated haemoglobin thereby increasing the level of total haemoglobin in diabetic rats. This could be due to the result of improved glycaemic control produced by ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg.

The body weight was decreased in Streptozocin diabetic rats. ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg increases the body weight in Streptozocin induced diabetic rats. The ability of ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/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).Lowering of serum lipids concentration through diet or drug therapy seems to be associated with a decrease in the risk

of vascular disease.The abnormal high concentration of serum lipids in diabetic subject 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 Streptozocin-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 *SeendhilSarkkarai* at a dose of 100mg/kg and 200mg/kg in Streptozocin induced diabetic rats.

The effect of ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg on diabetic hypertriglyceridemia could be through its control of hyperglycaemia. This is in agreement with the facts that:

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

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

The level of HDL-cholesterol was decreased in diabetic rats when compared with normal rats.Our results clearly show that the level of HDL-cholesterol was increased in Streptozocin induced diabetic rats when treated with ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg. These results suggest that ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg has protective effect against Streptozocininduced diabetes and its complications.

As reported earlier (Welihinda et al., 1986) in the current study also the liver glycogen content was reduced significantly in diabetic control as compared to non-diabetic control. Treatment with ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg prevented this alteration in glycogen content of liver tissue, but could not normalize the content of glycogen of the non-diabetic control. This prevention or depletion of glycogen in liver is possibly due

to either stimulation of insulin release from β-cells (Lolitkar et al., 1966) or due to the insulin mimetic activity of some components of the plants resulting in direct peripheral glucose uptake.

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)The present study also had similar results. Treatment with ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg significantly increased the hexokinase, Glucokinase activity and glucose-6-phosphate level in the liver, indicating an overall increase in glucose influx thus ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg seems to have an overall effect of increase in glucose utilization.

Oxidative stress is an imbalance between reactive oxygen species and the antioxidant defense mechanisms of a cell or tissue, which leads to lipid peroxidation, DNA damage, and the inactivation of many enzymes. The enzymatic antioxidant defense system is the natural protector against lipid peroxidation that includes superoxide dismutase, catalase and glutathione peroxidase. Reduced activities of these enzymes in the tissue of streptozotocin toxic rats were observed in our study. Superoxide dismutase protects against the superoxide radical (O_2^-), which damages the membrane and its biological structure. Catalase primarily decomposes hydrogen peroxide to H_2O at a much faster rate, sharing this function with glutathione peroxidase. Glutathione peroxidase may play an important role in the removal of lipid hydroperoxides. The balance between these enzymes is important for the efficient removal of oxygen radicals from tissues. Therefore, reduction in the activity of these enzymes may result in a number of deleterious effects due to the accumulation of superoxide radicals and H_2O_2. Significant increases in the activities of these enzymes were observed on ***SeendhilSarkkarai*** at a dose of 100mg/kg and 200mg/kg administration.

CONCLUSION

SeendhilSarkkarai at a dose of 100mg/kg and 200mg/kg has protective effect against Streptozotocin induced diabetes and its complications. Hence *seendhilsarkkarai* of Tinospora cordifolia is proved to be the promising medicinal plant which can be used as an adjunct to drug for the management of diabetes mellitus, dyslipidemia and effect of anti- oxidant enzymes in metabolism.

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