HYPOLIPIDEMIC EFFECT OF CITRULLUS COLOCYNTHIS SEED POWDER IN ALLOXAN INDUCED DIABETIC RATS

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Abstract

This study was undertaken to investigate the effect of Citrullus Colocynthis on blood glucose and some other Lipid biochemical parameters in alloxon induced diabetic rats. The aqueous extract treatment(300mg/kg body weight) was given for 22 days. Effect of this plant on the level of Glucose, total cholesterol, tri-glycerides, phospholipids, free fatty acids, LDL and HDL cholesterol and the enzyme activities like lipase, acetylcoA carboxylase, HMG coA reductase in serum and liver were evaluated. The Plant and Glibenclamide treated rats significantly reduce the elevated levels of Sugar, Cholesterol and other lipids in serum and liver. So these results indicate that Citrullus Colocynthis posse's hypolipidemic effect. (*Journal of International Dental and Medical Research 2009; 2: (3), pp. 105-109)*

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Introduction

Diabetes Mellitus is the heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (1). India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 (2). Along with hyperglycemia and abnormalities in serum lipids, diabetes is associated with micro and macro vascular complications, which are the major causes of morbidity and death. Management of diabetes without any side effects is still a challenge to the medical system. This leads to increasing demand for natural products with antidiabetic activity and less side effects. Therefore investigation from the medicinal plants has become more important because India has a rich history of using various potent herbs and herbal component for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes and reported in numerous scientific journals.

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Materials and Methods

Plant Material:

The fresh plant seeds of citrullus colocynthis was collected from perambalur area, Tamilnadu, was identified and authenticated from Botanist Dr. V.Kumaresan, Department of Biotechnology, Thanthai Hans Roever College, Perambalur. The seeds were dried and powdered in grinding mill.

Preparation of Extracts:

The Seeds were pulverized in grinder. 200gm of powdered seeds was poured in 400ml of distilled water for 1 hour at room temperature with continuous shaking. The mixture was filtered using sterile gauze. A fresh filtrate was used for treatment.

Animals:

Male Albino rats weighting about 150-200 gm obtained from the Eaisma Institue, Karur were used for the study. The rats were kept in the animal house at room temperature of 15 – 30 0 , fed with commercial food ad libitum and had free access to water. Animal studies in the work have been strictly performed as per the institutional of Animal Ethical Committee, Govt of India, and New Delhi.

Induction:

Alloxan Monohydrate was used to induce diabetes mellitus in normoglycemic rats. Animals were allowed to fast for 16 hours and were injected intraperitonially with freshly prepared alloxan monohydrate in normal saline in a dose of 120mg/kg body weight (3). Blood Glucose was measured after 72 hours by one touch glucometer and it was confirmed by testing for Glucosuria using Glucose indicator sticks. Rats showing fasting blood glucose levels > 200 mg/dl were selected for the study.

Experimental Design:

The animals were divided into 5 groups of six animals in each group.

Group 1: Normal Control.
Group 2: Normal Control + Plant treatment (300mg/kg body wt).
Group 3: Diabetic Control.
Group 4: Diabetic Control + Plant treatment.
Group 5: Diabetic Control + Glibenclamide treatment (0.5mg/kg body wt).

Biochemical Parameters:

The Blood Glucose level was determined by the method of sasaki et al.,(4). The Hemoglobin level was analysed by Cyanmethemoglobin method of drabkin and Austin. The Glycosylated Haemoglobin was measured by the method of Nayak and Patabiraman. Plasma insulin level was assayed by ELISA kit using human insulin as standard. The free fatty acid was determined by the method of Hron and Menahan (5-7). The cholesterol was estimated by Zak's method. Triglycerides was determined by the method of Rice (8). The Phospholipids was estimated by Bartlette Method (9). HDL and LDL were measured by the method of Burstein and Scholnick (10). The Lipase, HMGcoA reductase and acetylcoA carboxylase was determined by enzymatic kit method.

Statistical Analysis:

The values are expressed as mean ± Standard Deviation for six rats in each Group. All other data were analysed with SPSS/16.0 student software. Hypothesis testing method included one-way analysis of variants(ANOVA) followed by Post Hoc testing performed with least significant difference (LSD) test. The P value of less than 0.05 was considered to indicate statistical significance.

Group	Glucose (mg/dl)	Glycosylated Haemoglobin (mg/g/dl)	Haemoglobin (g/dl)	Insulin	Urine Sugar
Normal	89.33 ± 2.16	4.15 ± 0.15	11.3 ± 0.90	92.16 ± 6.49	Nil
Normal + CC.Aqt	93.50 ± 5.57^{a}	4.23 ± 0.20^{a}	10.0 ± 0.48^{a}	87.00 ± 19.43^{a}	Nil
Diabetic Control	228.00 ± 5.66^{b*}	10.23 ± 0.38^{b*}	7.73 ± 0.57^{b*}	48.18 ± 12.64^{b*}	+++
Diabetic + CC.Aqt	92.60 ± 2.16^{cd*}	4.95 ± 0.15^{cd*}	9.8 ± 0.29^{cd*}	83.56 ± 2.39^{cd*}	+
Diabetic + Glibenclamide	109.17 ± 16.25^{c*}	5.90 ± 0.88^{c*}	12.66 ± 1.65^{c*}	91.66 ± 6.25^{c*}	+

Table 1. Effect of Citrullus Colocynthis extract on the levels of Glucose, Glycosylated Haemoglobin, Haemoglobin, Insulin and urine sugar in Serum of alloxan Diabetic rats.

Group	Cholesterol (mg%)	LDL (mg%)	HDL (mg%)
Normal	156.16 ± 2.63	79.33 ± 1.21	61.66 ± 1.47
Normal + CC.Aqt	156.50 ± 3.39^{a}	72.83 ± 1.32^{a*}	62.16 ± 3.52^{a}
Diabetic Control	330.50 ± 31.86^{b*}	178.16 ± 1.60^{b*}	18.66 ± 2.16^{b*}
Diabetic + CC.Aqt	169.66 ± 6.34^{cd*}	84.00 ± 6.13^{cd*}	58.36 ± 5.98^{cd*}
Diabetic + Glibenclamide	161.66 ± 21.60^{c*}	79.01 ± 1.41^{c*}	56.38 ± 1.23^{c*}

Table 2. Effect of Citrullus Colocynthis extract on the levels of serum, cholesterol ,LDL, HDL level in control and experimental rats.

Group	Triglycerides (mg%)	free fatty acids (mg%)	Phospolipids (mg%)
Normal	119.50 ± 1.04	10.75 ± 3.06	167.17 ± 29.32
Normal + CC.Aqt	118.01 ± 2.16[a]	10.40 ± 3.23[a]	167.39 ± 27.38[a]
Diabetic Control	197.83 ± 11.51[b*]	23.33 ± 0.65[b*]	335.10 ± 30.82[b*]
Diabetic + CC.Aqt	117.50 ± 1.87[cd*]	10.41 ± 2.27[cd*]	203.33 ± 15.88[cd*]
Diabetic + Glibenclamide	105.83 ± 17.44[c*]	11.46 ± 1.33[c*]	190.36 ± 21.67[c*]

Table 3. Effect of Citrullus Colocynthis extract on the levels of serum, triglycerides, free fatty acids, phospolipids level in control and experimental rats.

Group	Lipase (IU/L)	AcetylcoA carboxylae (IU/L)	HMGcoA reductase (IU/L)
Normal	0.76 ± 0.35	7.25 ± 0.75	1.01 ± 0.25
Normal + CC.Aqt	0.77 ± 0.44[a]	7.36 ± 0.78[a]	1.08 ± 0.40[a]
Diabetic Control	2.90 ± 0.04[b*]	3.46 ± 1.04[b*]	0.32 ± 0.11[b*]
Diabetic + CC.Aqt	1.08 ± 0.30[cd*]	7.20 ± 0.62[cd*]	1.00 ± 0.52[cd*]
Diabetic + Glibenclamide	0.96 ± 0.23[c*]	7.81 ± 0.78[c*]	1.13 ± 0.36[c*]

Table 4. Effect of Citrullus Colocynthis extract on the levels of Serum ,lipase, acetylcoA carboxylase and HMGcoA reductase in control and experimental group of rats.

Group	phospholipids (mg/dl)	triglycerides (mg/g/dl)	free fatty acids (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal	166.83 ± 29.21	123.80 ± 11.32	9.75 ± 3.06	40.66 ± 9.60	66.83 ± 12.75
Normal + CC.Aqt	169.80 ± 27.63[a*]	120.61 ± 9.68[a*]	8.56 ± 2.02[a*]	40.83 ± 4.91[a]	69.16 ± 24.16[a*]
Diabetic Control	90.10 ± 61.53[b*]	210.10 ± 5.57[b*]	4.10 ± 1.30[b*]	23.16 ± 4.99[b*]	146.50 ± 6.31[b*]
Diabetic + CC.Aqt	161.00 ± 41.97[cd*]	131.75 ± 6.31[cd*]	8.61 ± 2.29[cd*]	41.16 ± 7.52[cd*]	76.66 ± 4.13[cd*]
Diabetic + Glibenclamide	165.56 ± 21.84[c*]	127.01 ± 4.56[c*]	9.88 ± 1.67[c*]	42.46 ± 3.32[c*]	71.41 ± 6.44[c*]

Table 5. Effect of Citrullus Colocynthis extract in liver phospholipids, triglycerides, free fatty acids, HDL and LDL cholesterol levels in control and experimental rats.

Group	lipase (IU/L)	acetylcoA carboxylase (IU/L)	HMGcoA reductase (IU/L)
Normal	0.70 ± 0.31	7.01 ± 0.68	0.90 ± 0.25
Normal + CC.Aqt	0.76 ± 0.44[a*]	7.28 ± 0.82[a*]	1.02 ± 0.50[a*]
Diabetic Control	0.15 ± 0.02[b*]	2.58 ± 0.74[b*]	0.33 ± 0.15[b*]
Diabetic + CC.Aqt	0.65 ± 0.45[cd*]	7.46 ± 0.91[cd*]	0.82 ± 0.40[cd*]
Diabetic + Glibenclamide	0.72 ± 0.31[c*]	7.16 ± 0.79[c*]	0.95 ± 0.37[c*]

Tab. 6 Effect of Citrullus Colocynthis extract in liver lipase, acetylcoA carboxylase, HMGcoA reductase activities in control and experimental rats.

Values are given as mean ± SD of six rats from each group
Values are statistically significant at *P<0.05
a-> Normal + Plant treated rats were compared with Normal Rats.
b-> Diabetic Rats were compared with Normal Rats.
c-> Plant treated Diabetic Rats were compared with Diabetic Rats.
d->Glibenclamide treated Diabetic Rats were compared with Diabetic Rats.

Results

Treatment with citrullus colocynthis aqueous extract on blood glucose level, insulin, Hb, HbA1C and Glycogen level are depicted in Table-1. The rats exposed to alloxan developed diabetes as evident from the significant elevation in blood glucose and HbA1C as compared to normal control rats. The administration of plant extract produced the significant reduction in blood glucose and HbA1C level. The insulin and Hb levels in diabetic control rats were decreased significantly compared to normal rats. In Diabetic treated groups(4 and 5) insulin and Hb levels are increased.

Table-2 shows the levels of total cholesterol, LDL and HDL levels of serum in control and diabetic

rats. The results revealed that there was a significant increase in total cholesterol and LDL level in diabetic rats. But the HDL level was reduced in diabetic control. The administration of the plant extract and the drug treatment shows the levels were reaches the near normal values.

The levels of triglycerides, free fatty acids and phospholipids are depicted in Table-3. The elevated levels of TG, FFA and phospholipids in diabetic rats were reduced after the treatment.

Table-4 and Table-6 shows the changes in the activities of lipase, acetylcoA carboxylase and HMGcoA reductase in serum and liver of control and experimental rats. The activity of lipase was increased in both serum and liver. In rats treated with the plant extract there was a reduction in the lipase activity. The reduced activities of acetylycoA carboxylase and HMGcoA reductase in diabetic control reach the normal value after the treatment of the plant extract.

Table-5 shows the effect of phospholipids, TG, FFA, HDL and LDL levels in liver of control and experimental rats. The elevated levels of triglycerides and LDL levels were reaches the normal after the plant treatment. The Phospholipids, FFA and HDL level were decreased significantly in diabetic control rats. Treatment with citrullus colocynthis, the level was significantly increased and reaches the near normal value.

Discussion

Diabetes Diabetes Mellitus is possibly the world largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases (11).

Traditional plant medicines are used throughout the world for a range of diabetic complication. The study of such medicines might offer diabetic complication. The study of such medicine might offer a natural key to unlock a diabetogenist's pharmacy for the future.

In animals, in can be induced by partial pancreatectomy or by the administration diabetogenic drugs such as alloxan, streptozotocin and anti-insulin serum (12). Alloxan, a beta cytotoxin causes a massive destruction of beta cells of the islets of langerhans resulting in reduced synthesis and release of insulin (13). It is well established that sulphonyl ureas produce hypoglycemia by increasing the secretion of insulin from pancreas.

The antidiabetic effect of citrullus colocynthis aqueous extract could be linked to more than one mechanism, it includes the stimulation of beta cells and subsequent release of insulin and activation of the insulin receptors. The plants have an antihyperglycemic action, may be by potentiation of pancreatic secretion of insulin, which was clearly evidenced by increased level of insulin in diabetic rats, treated with plant extract. In this context, a number of other plants have also been reported (14).

HbA1C was found to increase in patients with diabetes mellitus to about 16% (15) and the amount of increase is directly proportional to the fasting blood sugar level (16). The citrullus colocynthis reduces the elevated HbA1C level in diabetic rats.

In this study, we have also observed an increase in total cholesterol, TG and FFA in alloxan induced diabetic rats. Hyperlipidemia is a recognized consequence of diabetes mellitus (17). Diabetes induce hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization glucose(18). The ability of aqueous extract of citrullus colocynthis reduces the lipid level in diabetic rats.

Cardio vascular disease and stroke are the major complication in diabetes. This is due to increased LDL cholesterol, which further undergoes oxidative modification in the presence of free radicals leads to atherosclerosis and vascular damage. A decrease in LDL with raised HDL cholesterol plays an important role against these complications.

Insulin plays a key role in the metabolism of lipids apart from its regulation of carbohydrate metabolism. During diabetes, enhanced activity of lipase increases lipolysis and releases more fatty acids into the circulation (19). In this study we have observed an increase in lipase in alloxan induced rats. After the treatment the level was reduced.

AcetylcoA carboxylase is the key enzyme whose induction in beta cells may contribute to glucolipotoxicity. It catalyses an essential step in fatty acid synthesis in lipogenic tissues, this is usually a minor pathway of glucose usage in beta cells (20). However malonylcoA, the immediate product of this enzyme may play a signaling role in glucose stimulated insulin secretion (21). In alloxan induced diabetes, which causes abnormal metabolism of ketone bodies and is known to result in decreases of acetylcoA carboxylase (22). After the plant and drug treatment the levels were significantly reaches the normal.

HMGcoA reductase is a rate limiting enzyme in cholesterol synthesis. STZ induced diabetes in rats results in a marked decrease in the HMGcoA reductase in the liver (23). The role of insulin in promoting the synthesis of this enzyme in the liver

and the serum has been appreciated , although other work (24) has indicated that increases in enzyme during diabetes. In our study the reduced HMGcoA reductase was increased after the plant treatment.

Conclusions

From our study it is concluded that the aqueous extract of citrullus colocynthis seed powder exhibit hypolipidemic activity. This effect may be due to the presence of tannin, saponin, and flavanoids and other constituents of that plant.

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