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Efficacy of Methanolic Extract of *Costus Igneus* Rhizome on Hypoglycemic, Hypolipidimic Activity in Streptozotocin (STZ) Diabetic Rats and HPTLC Analysis of Its Active Constituents

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***Abstract*—The study was aimed to investigate the antihyperglycemic and hypolipidemic activities of methanol extract of Costus igneus rhizome (MECiR) in streptozotocin (STZ) induced diabetic albino rats. MECiR at a fixed dose range of 100, 200 mg/kg were orally administered as a single dose per day to diabetes induced rats for a period of 30 days. The effect of MECiR on blood glucose, glycosylated Hb (HbA1c) and serum lipid profile (Total cholesterol (TC), Triglyceride (TG), Very low density lipoprotein (VLDL), Low density lipoprotein (LDL), High density lipoprotein (HDL)) in plasma were measured in normal and diabetic induced rats. The results showed that fasting blood glucose, serum TC, TG, LDL, VLDL, levels were significantly ($p<0.05$) decreased, whereas serum HDL, level was significantly ($p<0.05$) increased in the diabetic rats. The dosage rate of 200 mg/kg is more effective than that of 100 mg/kg. Our investigation thus shows that MECiR has potent antidiabetic and hypolipidemic effects in STZ induced diabetic albino rats and found comparable to tested standard reference drug glibenclamide**

Keywords- Costus igneus; HPTLC; Hypoglycemic; Hypolipidimic

I. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion and or action [1]. Diabetes is becoming the third killer of humankind, after cancer and cardiovascular disease, because of its high prevalence, morbidity and mortality[2]. The presence of DM confers increased risk of many devastating complications such as cardio vascular disease, peripheral vascular disease complications such as coronary artery disease, stroke, neuropathy, renal failure, retinopathy amputations and blindness. Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas are available for the treatment of diabetes. The main disadvantages of the currently available drugs are that they have to be given throughout the life characterized with side effects [3]. But many traditional treatments have been recommended in the complementary and alternative system of medicine for the treatment of diabetes. Based on the WHO recommendation hypoglycemic agents of plant origin used in traditional medicine are of more important. Plant drugs and herbal formulation are frequently considered to be less toxic and free from side effects than synthetic one [4].

The mechanism of most of the herbals used to treat diabetes has not yet been defined. It has been attributed that the antihyperglycemic effect of these plants is due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent process. Hence treatment with herbal drugs has an effect on protecting beta cells and smoothing out fluctuation in glucose level. The present study was conducted to investigate the active compounds, antidiabetic, hypolipidemic and hepatic function enzymes effect of *MECiR* in streptozotocin induced diabetic albino rats.

II. MATERIALS AND METHODS

A. Animals

Albino Wister rats (150-200 g) were obtained from Sri Venkateshwara Enterprises, Bangalore (237/CPCSEA). The animals were housed in an air-conditioned room with controlled temperature and humidity, and they were fed with standard rat feed pellets that were supplied by Kamadhenu agencies (Bangalore, India) and filtered water *ad libitum*. Animals that underwent fasting were deprived of food for > 16 hrs, but they were allowed free access to water. Ethical clearance for the handling of experimental animals was obtained from the Institute of Animal Ethics Committee (265/CPCSEA).

B. Extraction and Active compound isolation

1) Plant material: The fresh *Costus igneus* (N.E.Br)(Costaceae family) rhizome was collected from Periyar Maniammai University nursery in the month of April (2008). Plant was identified, confirmed and authenticated by Rapinant Herbarium, St. Joseph's College, Trichy, Tamil Nadu, South India. All chemicals and reagents certified analytical grade purchased from Qualigens, Himedia and Loba Chemicals.

2) Extraction: Coarse powder from air-dried rhizome was subjected to a successive solvent extraction method using solvents of increasing polarity (petroleum ether, hexane, and

methanol, followed by water). The solvents were then distilled, evaporated and vacuum dried.

3) Phytochemical analysis: All extracts from the rhizome of *Costus igneus* (methanol, petroleum ether, water and hexane) underwent preliminary phytochemical analysis to identify their active components. The presence or absence of different phytoconstituents, e.g., triterpenoids, steroids, alkaloids, sugars, tannins, glycosides and flavonoids were detected by Mallikharjuna method [5].

4) Isolation and identification of Active compound: Plant active constituents responsible for anti diabetic properties were isolated by High Performance Thin Layer Chromatography (HPTLC). Acid hydrolysis was carried out on vacuum dried concentrated ethanol extract of *Costus igneus* to liberate aglycones, if any glycosides were present. The concentrates were spotted on activated TLC plates of silica gel F 254 of 0.5 mm thickness coating. The plates (10 X 10) were developed with solvent system Toluene: Ethyl acetate: Acetic acid: methanol (2:7:0.25:0.25) to elute quercetin. The developed plates were air dried and detected by 20% antimony chloride in chloroform, which was sprayed and dried in a chromatographic oven obtained at 105 ºC for 10 min. The resolution bands were obtained and retardation factor (Rf) value were calculated.

C. *Antidiabetic study*

1) Induction of diabetes mellitus in rats: Diabetes was induced by injecting streptozotocin (STZ) (Sigma, USA) at a dose of 40mg/kg bodyweight (bw) in 0.1M cold citrate buffer of pH 4.5, interaperitoneally. STZ- injected animals exhibited severe glycosuria and hyperglycemia and rats were stabilized over a period of 7 days. On set of diabetes was confirmed in the experimental rats by measuring blood glucose concentration at 96 h after injection with STZ. The rats with blood glucose level above 250 mg/dl were considered to be diabetic and were used for the experiment. Control rats were administrated with citrate buffer (pH 4.5).

2) Experimental design: Animals were grouped in to five groups having six rats in each group. Group I served as a control; Group II had STZ-treated surviving diabetic rats; Group III had the STZ-induced diabetic rats treated with M*ECiR* (100mg/kg bw/day); Group IV had the STZ-induced diabetic rats treated with M*ECiR* (200mg/kg bw/day); Group V served as a positive control and received Glibenclamide (5mg/kg/bw) for 30 days by oral administration. Rats were sacrificed at the termination of the experiment i.e on the 30th day and the blood samples were collected and analyzed for selected biochemical parameters.

At the end of the treatment, blood was collected by cardiac puncture and serum was separated by centrifugation at 2500 rpm and was used for the estimations of various parameters such as Serum glucose [6], liver glycogen, [7], HbA1c and lipid profile (Cholesterol, Triglycerides [8], Low density lipoprotein (LDL) [9], High density lipoprotein (HLD) [10] were analyzed.

The parameter values were analyzed by one way ANOVA analysis. All the results were expressed as mean ± SD for six rats in each group and $P<0.05$ was considered as statistically significant.

III. RESULTS

A. *Phytochemical analysis*

Compounds of different polarity from dried rhizome powder of *Costus igneus* were extracted using solvents. These extracts were subjected to preliminary phytochemical screening for the presence of different chemical group. Of all the extracts analyzed methanol extract was found to contain the highest number of phytochemicals such as Tannins, Saponin, Flavonoids, Terpenoids, Cardiac glycosides (Table I) and naturally occurring phenolic compound like quercetine was isolated by HPTLC (Rf 0.72) conformed to Standard quercetine (Rf 0.72) (Fig.1).

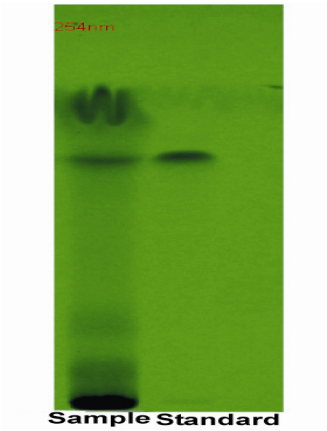

Figure 1. Isolation of quercetin from Costus igneus rhizome

B. *Biochemical parameters*

Serum glucose, and HbA1c levels in rats in different groups (Table II) were significantly ($p<0.05$) higher in group II (Diabetic control) compared with group I (Normal control). On other hand the level of these parameters were significantly ($p<0.05$) decreased in group III (*MECiR* 100 mg/kg), IV (*MECiR* 200 mg/kg) and group V (Glibenclamide). The results suggest that *MECiR* has an antidiabetic effect but does not lead to hypoglycemic activity at the tested dose.

TABLE II. EFFECT OF SECIR ON GLUCOSE AND HBA1C LEVELS IN STZ-INDUCED DIABETIC PRODUCTIVE RATS ON THE 30TH CONSECUTIVE DAY OF ADMINISTRATION.

Groups	Glucose	HbA1C
Normal	91.55±1.269	5.38±0.35
Diabetic control	285.13±1.315 [a]	7.275±0.189 [a]
M*ECiR* (100mg/kg)	124.5±3.5 [a,b]	6.1±0.141 [a,b]
M*ECiR* (200mg/kg)	102.6±1.7 [b,c]	5.37±0.12 [b,c]
Glibenclamide	106.5±1.291 [a,b,c,d]	5.85±0.129 [a, b,c,d]

Values represent mean ± SD (n=6); comparisons between groups are as follows: a: Group I vs. Groups II, III, IV, V; b: Group II vs. Groups III, IV, V; c: Group III vs. Groups IV, V; d: Group IV vs. Group V. Statistical significance was considered to be [a]p< 0.05, [b]p< 0.05, [c]p< 0.05, [d]p< 0.05

The lipid profile in control and experimental rats are depicted in Table 3**.** In STZ-induced diabetic rats (group II), there was a significant ($p<0.05$) increase of total cholesterol, triglycerides, LDL, VLDL and significant ($p<0.05$) decrease in HDL cholesterol in serum compared to normal control

(group I). Administration of *MECiR* (group III and IV) and glibenclamide (group V) for 30 days has significantly ($p<0.05$) lowered the high values back to normal levels of total cholesterol, triglycerides, LDL, VLDL and significantly ($p<0.05$) increase in HDL cholesterol. This indicates that the *MECiR* had hypolipidimic effects on lipid metabolism in diabetic induced rats.

IV. Discussion

Diabetes mellitus is a serious chronic disease. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients [11],[12],[13]. Antihyperglycemic potency of the methanol extract of *Costus igneus* rhizome (M*ECiR*) in diabetic induced rats has been indicated here by estimation of fasting blood glucose levels, as the important basal parameter for monitoring of diabetes and the dosage of 200 mg/kg is more effective than that of 100 mg/kg. The increased level of glycosylated Hb(HbA1c) observed in diabetic induced rats might be due to decreased formation of Hb. It has been reported that in diabetic subjects the total haemoglobin level is much lower than the normal level and increased levels of HbA1c. Earlier report states that during diabetes mellitus, excess of blood glucose reacts with hemoglobin leading to the formation of HbA1c. The level of HbA1c is always monitored as a reliable index of glycemic control in diabetes [14]. Elevated levels of HbA1c and reduced levels of Hb observed in our study reveal that diabetic animals had prior high blood glucose level. Administration of *MECiR* (100, 200mg/kg bw/day) has lowered the elevated HbA1c levels to near normal level. It has already been reported that decreased liver glycogen level is due to insulin deficiency and associated glycogenolysis [15]. The possibility of restoring liver glycogen level in STZ-induced diabetic rats by the *MECiR* may be attributed to increased insulin secretion and or reactivation of glycogen synthase enzyme system. Diabetes is also associated with hyperlipidemia [16]. The levels of TC and TG have been decreased significantly in diabetic induced rat after the *MECiR* supplementation. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis, which are under the control of insulin [17]. The *MECiR* supplementation also results to the significant attenuation in the level of serum HDL towards the control level, which again strengthens the hypolipidemic effect of this extract. There are report that other medicinal plants have hypoglycemic and hypolipidemic effects that could prevent or be helpful in reducing the complications of lipid profile seen in some cases of diabetes in which hyperglycemia and hypercholesterolemia coexist [18].

In present study quercetin was isolated as a active principle of *MECiR* it may contribute to the reduction in blood glucose level and also lipid profile. This correlates with the previous report Nuraliev & Avezov [19] on the effect of quercetin in decreasing the level of cholesterol VLDL and LDL levels.

V. Conclusion

The present study indicates that *MECiR* may have beneficial effects as antidiabetic and antihyperglycemic agents and also warrants further studies to isolate and characterize potent molecules for the treatment of diabetes mellitus and its lipids associated complications.

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References

[1] A.F. Amos, D. Mc Carty and P. Zimmet, "The rising global burden of diabetes and its complications: Estimates and projections to the year 2010," Diabet.Med, 14,81-85,1997.

[2] W.L. Li, H.C. Zheng, J. Bukuru and N. De kimpe, "Natural medicine used in the traditional Chinese medical system for the therapy of diabetes," J. Ethanopharmacol. 92, 1-21,2004.

[3] E.M. Halim, "Effect of Coccinia indica and Abroma augusta on glycemia, lipid profile and on indicators of end organ damage in streptozotocin induced diabetic rats," Indian. j. Clin. Biochemistry., 18,54-63,2003.

[4] WHO Study Report. Diabetes mellitus, WHO Tech Rep Se.r. 727,1-113, 1985.

[5] P. B. Mallikharjuna, L. N. Rajanna, Y. N. Seetharam, and G. K. Sharnabasappa, "Phytochemical studies of Stychnos potatorum L.- A Medicinal plant". E-Journal of Chemistry. 4(4), 510-518,2007.

[6] P .Trinder," Enzymatic method of glucose determination in serum," Ann. Clin.
Biochem.,6, 24,1969.

[7] S. Seifter, S. Dayton, B. Novic and E. Muntwyler, "The estimation of glycogen with anthrone reagent," Arch. Biochem, 25, 191-200,1950.

[8] P. Fosseti and L. Prencipe, "Enzymatic determination of triglycerides," Clin. Chem., 28, 2077, 1982.

[9] D. Steinberg, "Metabolism of lipoproteins at the cellular level in relation to atherogenesis. In lipoproteins, atherosclerosis and cornary heart disease," Elsevier. North Holnd,1(2),31-48,1981.

[10] Arcol, "Separation of high density lipoproteins and determination of cholesterol and phospholipids bound to these fractions European Atherosclerosis Society," European Heart J., 15: 121-124,1989.

[11] C. Abraira, J.A. Colwell, F.Q. Nuttall, C.T. Sawin, N.J Nagel and J.P Comstock, "Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). Results of the feasibility trial. Veterans Affairs Cooperative Study in Type II Diabetes," Diabetes Care, 18,1113-1123,1995.

[12] Y. Ohkubo, H. Kishikawa, E. Araki, T. Miyata, S. Isami, and S. Motoyoshi, "Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulindependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res. Clin. Pract, 28, 103–117,1995.

[13] R.A. DeFronzo, "Pharmacologic therapy for type 2 diabetes mellitus," Ann. Intern. Med, 131, 281–303,1999.

[14] K.H. Gabbay, "Glycosylated hemoglobin and diabetic control," New Eng J Med, 95, 443-454,1976.

[15] V. Vats, S.P. Yadav and J.K. Grover, "Ethanolic extract of Ocimum sanctum leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats," J Ethnopharmacol, 90,155-160,2004.

[16] M. De Sereday, C. Gonzalez, D. Giorgini, B.J. DeLoredo, C. Cobenas, C. Tebone, and C. Libman, "Prevalence of diabetes, obesity,

hypertension and hyperlipidemia in the central area of Argentina," Diabetes Metab, 30,335–339,2004.

[17] S.B. Sharma, A. Nasir, K.M. Prabhu, P.S. Murthy and G. Gev, "Hypoglycaemic and hypolipidemic effect of ethanolic extract of seeds of Eugenia jambolana in alloxan-induced diabetic rabbits," J. Ethnopharmacol, 85, 201-206, 2003.

[18] R.D. Sharma, T.C. Paghuram and T.S. Rao, "Effect of fenugreak seed on blood glucose and serum lipids in type I diabetic," European j. of clin.chem, 44, 301-306, 1990.

[19] I.N. Nuraliev and G.A. Avezov, "The efficacy of Quecertin in alloxan diabetes". Eks.Klin.Farmakol, 55, 42-44, 1992.

TABLE I. QUALITATIVE PHYTOCHEMICAL ANALYSIS IN DIFFERENT EXTRACTS OF *COSTUS IGNEUS* RHIZOME

Extracts	Tannin	Phlobatannin	Saponin	Flavonoid	Steroid	Terpenoids	Cardiac glycosides
Ethanol	+	+	+	+	+	+	+
Methanol	+	-	+	+	-	+	+
Hexane	-	-	+	-	+	-	-
Petroleum Ether	-	-	+	-	+	+	-

TABLE III. EFFECT OF SECIR ON LIPID PROFILE AT 30TH DAY

Groups	**Cholesterol**	**Triglycerides**	**HDL**	**LDL**
Normal	81.75±1.89	76±1.414	40±1.29	29.75±0.957
Diabetic control	122.88±1.31[a]	122.5±1.83[a]	20.5±1.29[a]	46.25±1.25[a]
M*ECiR* (100mg/Kg)	104±1.78[a,b]	95.1±2.4[a,b]	28.6±1.08[a,b]	43.3±1.96[a,b]
M*ECiR* (200mg/Kg)	92±2.36[a, b,c]	82.5±1.8[a, b,c]	35.7±1.08[a,b,c]	32.8±1.47[ab,c]
Glibenclamide	90.5±1.29[a, b,c]	86.5±1.29[a,b,c]	33.25±0.957[a ,b,c]	36.75±0.957[a,b,c]

Values represent mean ± SD (n=6); comparisons between groups are as follows: a: Group I vs. Groups II, III, IV, V; b: Group II vs. Groups III, IV, V; c: Group III vs. Groups IV, V; d: Group IV vs. Group V. Statistical significance was considered to be [a]p< 0.05, [b]p< 0.05, [c]p< 0.05, [d]p< 0.05