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Research Article

METHANOLIC EXTRACT OF *COSTUS IGNEUS* (N.E.Br.) ALLEVIATES DYSLIPIDEMIA IN DIABETIC RATS

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ABSTRACT

The present study was aimed at analyzing the hypolipidemic effects of methanol extract of *Costus igneus* leaves in streptozotocin-induced diabetic rats. In diabetes, dyslipidemia coexist quite often. Male diabetic rats were treated with 100 mg/kg/day of methanolic extract orally for 30 days. The experiment showed promising results by significantly decreasing cholesterol, triglycerides, free fatty acids and phospholipids in the liver, heart and kidney of diabetic treated rats. Lipoproteins restored normal levels in treated group, significantly reducing serum total cholesterol and increasing HDL cholesterol. Activity of lipoprotein lipase was enhanced in extract treated group. Glucose-6-phosphate dehydrogenase, LCAT and malic enzyme activities which were significantly lower in diabetic rats showed considerable increase in treated rats. Our studies indicate that methanolic leaf extract of *Costus igneus* exerts potent hypolipidemic effects in diabetic rats. Hence the plant may also be useful in the cure and management of secondary complications of diabetes.

Keywords: *Costus igneus*; MEC; Streptozotocin; HDL Cholesterol; Lipoprotein lipase; HMG CoA Reductase; Plasma LCAT.

INTRODUCTION

Diabetes mellitus is a major risk factor for the development of atherosclerosis. Patients with type 2 diabetes mellitus tend to experience premature, severe coronary atherosclerosis[1], and diabetes results in a two- to four fold increased risk of coronary heart disease[2]. Unregulated cholesterol levels lead to serious pathological conditions. It is widely understood that cholesterols, especially LDL cholesterol and its oxidized derivatives play an important role in the pathogenesis of atherosclerotic conditions. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics[3]. Insulin deficiency or insulin resistance is associated with hypercholesterolemia and hypertriglyceridemia[4]. Diabetes induction causes increase in the cholesterol, triglycerides, VLDL and LDL[5]. Coronary artery disease, as a result of premature atherosclerosis, is a major cause of death both in type I and II diabetes.

Recently, interest in substances of plant origin, which promote normalization of lipid metabolism under conditions of lipid pathology has increased. The protective effects of plant products are due to the presence of several components, which have distinct mechanisms of action; some of them are enzymes and proteins and others are low molecular weight compounds such as vitamins, carotenoids, flavonoids[6], anthocyanins and other phenolic compounds[7]. *Costus igneus (C. igneus),* common name 'Fiery Costus' or 'Spiral Flag' is a species of herbaceous plant in the Costaceae family. Our previous experiments proved significant hypoglycemic effect of this plant[8]. The hypoglycemic, hypocholesterolemic and anti atherosclerotic effects may be due to the presence of several components like triterpene saponins[9], individual triterpene acids[10], flavonoids[11] and certain other plant derived compounds which are predominant in the methanolic extract of *Costus igneus*[8].

MATERIALS AND METHODS

Plant material

C. igneus was collected from Konni, Pathanamthitta, Kerala, India. The plant was identified and authenticated by Dr. G. Valsaladevi, Curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, India. A voucher specimen was deposited at Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, India (Voucher No. KUBH 5791).

Preparation of Methanolic Extract of *Costus igneus* (MEC)

Extraction and preparation of crude extracts were carried out by cold percolation method at room temperature and by solvent evaporation. This helps in protection of any heat labile metabolite present in it.

Extraction was performed in 80% methanol. It was kept in cold room overnight. After centrifugation at 3000 rpm for 15 min the extract was treated with petroleum ether. Methanol was then removed via rotavapour (Speed Vac) at 40-50^{0}C under reduced pressure. It was then dried in a desiccator to get a brown sticky compound. 100 g fresh leaves yielded 1.681 g extract.

Chemicals

All the chemicals used were high quality analytical grade reagents. Streptozotocin was purchased from Sigma Aldrich Co., USA. One-touch glucometer was purchased from Bayer Diagnostics India Ltd. Solvents such as methanol, petroleum ether etc were purchased from Merk, India. All other chemicals used for the study were of analytical grade.

Animals

Male albino rats of Sprague- Dawley strain, with identical age and comparable weight, (150-180 g) used for the experiment were obtained from Animal House, Department of Biochemistry, University of Kerala, Thiruvananthapuram, India. Animals were housed in standard polypropylene cages. Cages were kept in an environment with controlled temperature (28-32°C), humidity (55–60%) and photoperiod (12:12 h) light–dark cycle and fed a standard diet supplied by Gold Mohur rat feed, Hindustan Lever Ltd and given water *ad libitum*.

Animal experimentation was conducted in accordance with the institutional ethical guidelines for the conduct of the experiments on laboratory animals as per CPCSEA rules [Sanction No: IAEC-KU-13/05-06-BC-AH (4)]. Animals were handled using the laboratory animal welfare guidelines[12]. They were weighed weekly and the food and water consumption were noted. The animals were also examined regularly for their physical activity.

Induction of diabetes: Rats were pretreated with streptozotocin (STZ) [60 mg/kg, i.p.] to induce diabetes. STZ–induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents[13]. STZ selectively destroys the pancreatic insulin secreting β cells, leaving less active cells and resulting in a diabetic state[13].

Because STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release, rats were treated with 20% glucose solution (5-10 ml) orally after 6 hr. The rats were then kept

for the next 24 hr with 5% glucose solution bottles in their cages to prevent hypoglycemia[14].

After specific time periods, experimental rats were sacrificed by euthanasia (intraperitoneal injection of ketamine at a dose of 60 mg/kg), and the blood and tissue samples were collected immediately.

Experimental Design

For evaluating the hypoglycemic activities of MEC, rats were divided into 3 groups, with 12 rats in each group.

Group I: Normal control (NC)
Group II: Diabetic control
Groups III: Diabetic+MEC at a dose of 100 mg/kg BW (MEC_{100})

The duration of the experiment was 30 days. MEC was dissolved in physiological saline and given orally by gastric intubation.

The tissue was extracted according to the procedure of Folch *et al.*, (1957)[15]. In order to check the hypolipidemic activity of MEC_{100} on lipid metabolism in diabetic rats, detailed studies were conducted to analyse various lipid components like cholesterol[16], free fatty acid[17], triglycerides[18], phospholipids[19], serum lipoproteins[20,21] and activities of various enzymes like lipoprotein lipase[22], HMG Co-A reductase[23], glucose-6-phosphate dehydrogenase[24], plasma LCAT[25], malic enzyme[26] etc. They were done according to the procedures described earlier.

Statistical analysis

The results were analyzed using a statistical program SPSS/PC+, Version 11.0 (SPSS Inc. Chicago, USA). One way ANOVA was employed for comparison among groups. Post-hoc multiple comparison tests of significant differences among groups were determined. Pair fed comparisons between the groups was made by Duncan's multiple range tests[27].

Correlation coefficient r (Pearson, Spearman) were used for statistical evaluation. All results were considered as statistically significant at $P \leq 0.05$.

RESULTS

Concentration of cholesterol

Diabetic rats showed a hike in the concentrations of cholesterol in liver, heart and kidney but administration of MEC_{100} lowered cholesterol concentration to almost normal levels (Table1).

Concentration of free fatty acid

Concentration of free fatty acid in liver, heart and kidney was elevated in group II where as it was brought to lower level in all tissues of group III (Table 2).

Concentration of triglycerides

In diabetic rats, there was significant increase of triglycerides in liver, heart and kidney, while it was lowered significantly in all tissues of group III (Table 3).

Concentration of phospholipids

Concentration of phospholipids was elevated in liver, heart and kidney of diabetic group, but administration of MEC_{100} lowered its concentration to almost normal levels (Figure 1).

Concentration ofserum lipoprroteins

In diabetic group lipoprotein concentration was altered when compared to normal controls. Lipoproteins restored normal levels in MEC_{100} treated group (Table 4). HDL cholesterol was raised to significant levels in treated animals.

Activity of lipoprotein lipase

Activity of lipoprotein lipase was diminished significantly in diabetic rats where as its activity was enhanced in heart and adipose tissue of MEC_{100} treated group (Table 5).

Activity of hmg co-a reductase

Activity of HMG Co-A Reductase in liver and intestine was lowered in diabetic rats, but attained normal levels in MEC_{100} treated group (Table 6).

Activities of glucose-6-phosphate dehydrogenase, plasma lcat & malic enzyme

Activities of glucose-6-phosphate dehydrogenase, LCAT and malic enzyme were significantly lower in diabetic rats than normal control rats where as their activities showed significant elevation in rats fed MEC_{100} (Table 7).

DISCUSSION

Several theories have been expounded for the atherogenic effects of diabetes. The abnormally high concentrations of plasma and hepatic lipids in diabetes is mainly due to an increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits hormone sensitive lipase[28]. The marked hyperlipidemia that characterizes the diabetic state is regarded as a consequence of the uninhibited actions of lipolytic hormones (glucagon & catecholamines) on the fat depots[29]. On the other hand, increased LDL-cholesterol may arise from glycosylation of lysyl residues of apoprotein B[29].

Administration of MEC_{100} lowered cholesterol, triglycerides, phospholipids and free fatty acids to near normal values. This study demonstrates that administration of MEC_{100} was able to alter the lipid metabolism by lowering the levels of lipid parameters in the serum and tissues. FFAs contribute to insulin resistance by inhibiting glucose uptake, glycogen synthesis, glycolysis, and by increasing hepatic glucose production[30]. FFAs also stimulate expression of gluconeogenic enzymes, including glucose-6-phosphatase[31]. Activity of malic enzyme was also restored to normal level in MEC_{100} treated rats. Ativity of HMG Co-A Reductase was decreased in diabetic rats, which is consistent with the findings of Nakayama and Nakagawa[32] where Hepatic HMG-CoA reductase activities were markedly reduced in STZ induced diabetic rats. This and other reports[32-35] establishes that streptozotocin-induced diabetes in rats results in a marked decrease in the total activity of HMG-CoA reductase in the liver. MEC_{100} treatment raised enzyme levels to normal levels.

MEC_{100} treated rats showed higher activity of lipoprotein lipase (LPL) in heart tissue and adipose tissue and higher activity of lecithin-cholesterol acyl transferase (LCAT) compared to diabetic control. It is reported that a deficiency in LPL activity in diabetics may contribute to significant elevation of triglycerides in blood and with insulin administration, LPL activity is elevated and leads to lowering of plasma triglyceride concentrations[36]. High LPL activity is associated with a high clearance rate of dietary fat and a high HDL level. HDL plays a central role in reverse cholesterol transport because it not only promotes the efflux of cholesterol from peripheral tissues but is also the major site for the esterification of cholesterol by LCAT. LCAT activity modulates cholesterol transfer from lipoproteins and cell membranes to HDL. Therefore, decreased activity of LCAT promotes the accumulation of free cholesterol at cell membranes, and of remnant lipoprotein in plasma, both factors being strongly related to atherosclerosis[37]. Administration of MEC_{100} could almost correct lipoprotein abnormalities, a well-known consequence of diabetes. From our previous studies, it was shown that MEC contained many phytochemicals such as steroids, triterpenoids, alkaloids, tannins, glycosides, saponins, and flavonoids.

The above findings clearly state that methanolic extract of *C. igneus* at a dose of 100 mg/kg BW can reverse the hyperlipidemic complications in the diabetics to a greater extent. Further studies on the structure activity relationships of the active components of this plant may provide leads for the development of a drug with enormous therapeutic possibilities.

Table 1: Concentration of Cholesterol

Groups	Liver	Heart	Kidney
	(mg cholesterol/100 g tissue)		
I (Control)	327.17±16.24	192.05±9.61	272.94±13.69
II (Diabetic)	452.72±21.06[a]	252.01±12.07[a]	305.32±15.25[a]
III (D+MEC$_{100}$)	338.12±16.83[b]	208.48±10.42[b]	291.57±14.55[b]

Values expressed as mean ± SEM, for n = 6.
a-Statistically significant when compared to group I at p < 0.05
b- Statistically significant when compared to group II at p < 0.05

Table 2: Concentration of free fatty acid

Groups	Liver	Heart	Kidney
	(mg free fatty acid/100 g tissue)		
I (Control)	254.25±12.71	251.69±12.08	180.38±8.27
II (Diabetic)	287.35±13.45[a]	284.55±13.75[a]	205.54±9.71[a]
III (D+MEC$_{100}$)	261.65±12.62[b]	259.57±12.59[b]	89.34±9.74[b]

Values expressed as mean ± SEM, for n = 6
a-statistically significant when compared to group I at p < 0.05.
b-Statistically significant when compared to group II at p < 0.05

Table 3: Concentration of Triglycerides

Groups	Liver	Heart	Kidney
	(mg glycerol/100g tissue)		
I (Control)	501.46±19.81	60.31±2.91	73.60±3.42
II (Diabetic)	583.54±22.35[a]	89.25±3.56[a]	81.55±4.03[a]
III (D+MEC$_{100}$)	518.50±21.74[b]	64.72±3.04[b]	74.54±3.68[b]

Values expressed as mean ± SEM, for n = 6
a-Statistically significant when compared to group I at p < 0.05.
b-Statistically significant when compared to group II at p < 0.05

Table 4: Concentration of Serum Lipoproteins

Groups	Total cholesterol	HDL-cholesterol	LDL+VLDL-cholesterol
	(mg serum lipoproteins/dl)		
I (Control)	70.94±3.51	45.41±2.12	25.53±1.25
II (Diabetic)	80.38±4.03[a]	39.53±1.72[a]	40.85±2.07[a]
III (D+MEC$_{100}$)	72.06±3.52[b]	43.95±2.07[b]	28.11±1.36[b]

Values expressed as mean ± SEM, for n = 6
a-Statistically significant when compared to group I at p < 0.05
b-Statistically significant when compared to group II at p < 0.05

Table 5: Activity of Lipoprotein lipase

Groups	Heart (µmol glycerol liberated/hr/g protein)	Adipose (µmol glycerol liberated/hr/g protein)
I(Control)	45.35±2.25	286.27±11.47
II (Diabetic)	38.56±1.91[a]	241.30±9.62[a]
III (D+MEC$_{100}$)	42.47±2.11[b]	264.53±11.07[b]

Values expressed as mean ± SEM, for n = 6.
a-Statistically significant when compared to group I at p < 0.05
b-Stastically significant when compared to group II at p < 0.05

Table 6: Activity of HMG Co-A Reductase[x]

Groups	Liver (Hmg coa/ mevalonate)	Intestine (Hmg coa/ mevalonate)
I (Control)	2.32±0.10	2.93±0.13
II (Diabetic)	4.08±0.17[a]	5.15±0.20[a]
III (D+MEC$_{100}$)	2.61±0.12[b]	3.03±0.14[b]

Values expressed as mean ± SEM, for n = 6 a-Statistically significant when compared to group I at p < 0.05
b-Statistically significant when compared to group II at p < 0.05.
[x]Units ([HMG Co A]/[mevalonate]. Lower the ratio, higher the activity)

Table 7: Activity of Glucose-6-phosphate Dehydrogenase*, Plasma LCAT[Φ]& Malic Enzyme[Ψ]

Groups	G-6-P Dhase (Units*/g protein)	Plasma LCAT[Φ] (estercholesterol/free cholesterol)	*Malic enzyme (Units[Ψ]/g protein)*
	(mg cholesterol/100 g tissue)		
I (Control)	120.61±5.81	71.29±3.05	1.82±0.07
II(Diabetic)	97.60±4.37[a]	48.23±2.12[a]	1.23±0.05[a]
III (D+MEC$_{100}$)	113.57±5.06[b]	67.39±2.03[b]	1.56±0.06[b]

Values expressed as mean ± SEM, for n = 6
a-Statistically significant when compared to group I at p < 0.05
b-Stastistically significant when compared to group II at p < 0.05
*The amount of enzyme which causes an increase in OD of 1.0/min
[Φ]Activity expressed as % increase in the ratio of ester cholesterol to free cholesterol during incubation
[Ψ]The amount of enzyme which causes an increase in OD of 0.1/min

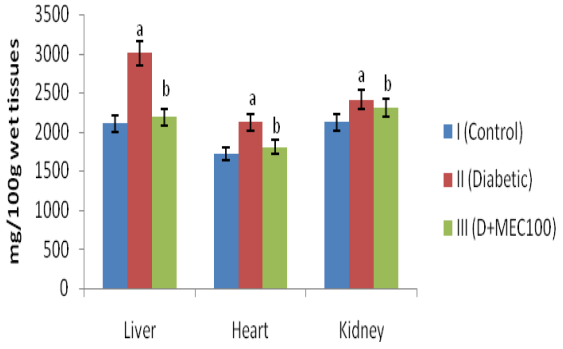

Fig. 1: Concentration of phospholipids

Values expressed as mean ± SEM, for n = 6
a-Statistically significant when compared to group I at p < 0.05
b-Statistically significant when compared to group II at p < 0.05

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