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Protective effect of *Coccinia indica* on changes in the fatty acid composition in streptozotocin induced diabetic rats

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The present study was undertaken to investigate the effect of *Coccinia indica*, an indigenous plant used in Ayurvedic Medicine in India, on blood glucose, plasma insulin, cholesterol, triglycerides, free fatty acids and phospholipids and fatty acid composition of total lipids in liver, kidney and brain of normal and streptozotocin (STZ) diabetic rats. Oral administration of the ethanolic extract of *Coccinia indica* leaves (200 mg/kg body weight, CLEt) for 45 days to diabetic rats decreased the concentrations of blood glucose, lipids and fatty acids, viz., palmitic, stearic, and oleic acid whereas linolenic and arachidonic acid and plasma insulin were elevated. These results suggest that CLEt exhibits hypoglycaemic and hypolipidaemic effects in STZ induced diabetic rats. It also prevents the fatty acid changes produced during diabetes. The effect of CLEt at 200 mg/kg body weight was better than that of glibenclamide.

1. Introduction

Fatty acids undergo changes during the process of injury, repair and cell growth [1]. Fars et al. found that there is an alteration in the erythrocyte membrane and plasma fatty acid composition in diabetic patients [2]. Previous results have also shown that there is a significant alteration in the fatty acid composition of serum and a variety of tissues in experimental diabetes [3]. The alteration of the membrane phospholipid composition appears to be responsible for the biochemical alterations produced during long term diabetes [4].

Coccinia indica W & A (Cucurbitaceae), commonly known as Little gourd and locally known as "Kovai", grows abundantly and wildly all over India. Mukherjee et al. showed that the aqueous and ethanolic extract of *Coccinia indica* leaves possess hypoglycemic activity [5]. These workers further showed that the active principles were water soluble, dialysable and gave positive tests for alkaloids. They have found that the alkaloids present in the ethanolic extract of *Coccinia indica* are Cephalandrine A and Cephalandrine B. Several years ago (1992) Hossain et al. examined the effect of *Coccinia indica* leaf preparation on 48 h starved normal male rats and showed that the leaf extract decreased the activity of the enzyme glucose-6-phosphatase [6]. Recently we have proved the antioxidant effect and also insulin stimulatory effect of *Coccinia indica* leaves from existing β-cells in diabetic rats [7, 8].

To our knowledge, no other biochemical investigations have been carried out on the effect of *Coccinia indica* in normal and streptozotocin diabetic rats on tissue fatty acid composition and lipids. Thus, the present investigation was carried out to study the effect of *Coccinia indica* leaf extract CLEt on fatty acid composition and lipids in normal and streptozotocin diabetic rats.

2. Investigations, results and discussion

2.1. Effect of Coccinia extract on blood glucose and plasma insulin

Table 1 demonstrates the levels of blood glucose and plasma insulin in normal and experimental animals at the end of 30 and 45 days. The level of blood glucose was significantly increased whereas the level of plasma insulin was significantly decreased in diabetic rats. The administration of CLEt at the dose of 200 mg/kg body weight showed a much more significant effect than 50 mg and 100 mg/kg body weight. In the CLEt treated groups, although a significant antihyperglycaemic effect was evident on 30th day of treatment, the decrease in blood sugar was highly significant on 45th day in the group treated with 200 mg/kg body weight. CLEt was more potent than glibenclamide.

As the effect of CLEt was most effective at a dose of 200 mg/kg body weight in 45 days treatment, the dose was selected for further biochemical studies.

2.2. Effect of CLEt on tissue lipids and fatty acid composition

Tables 2–6 show the changes in the level of tissue cholesterol, triglycerides, free fatty acids, phospholipids and alterations in the fatty acid composition in liver, kidney and brain of control and experimental rats. There was a significant elevation in tissue cholesterol, triglycerides, free fatty acids, phospholipids, palmitic acid (16 : 0), stearic acid (18 : 0) and oleic acid (18 : 1) in streptozotocin diabetic rats. In contrast, there was a significant decrease in the concentration of linolenic acid (18 : 3) and arachidonic acid (20 : 4) in tissues of diabetic rats. Oral administration of CLEt and glibenclamide significantly decreased the

Table 1: Effect of CLEt on the levels of blood glucose and plasma insulin in normal and experimental rats

Group	Blood glucose (mg/dl)		Plasma insulin (μU/ml)	
	30 days	45 days	30 days	45 days
1. Normal	75.32 ± 4.65^{a}	77.05 ± 4.87^{a}	14.15 ± 0.68^{a}	14.41 ± 0.70^{a}
2. Normal + CLEt	68.40 ± 3.25^{a}	65.30 ± 3.48^{a}	15.62 ± 0.62^{a}	16.00 ± 0.96^{a}
3. Diabetic control	262.32 ± 20.32^{b}	266.10 ± 21.00^{b}	4.20 ± 0.28^{b}	4.13 ± 0.26^{b}
4. Diabetic + CLEt (50 mg/kg)	241.60 ± 17.85^{b}	227.30 ± 18.61^{b}	4.36 ± 0.30^{b}	4.52 ± 0.35^{b}
5. Diabetic + CLEt (100 mg/kg)	172.35 ± 14.52^{b}	154.20 ± 12.31^{c}	5.23 ± 0.47^{c}	5.84 ± 0.43^{c}
6. Diabetic + CLEt (200 mg/kg)	98.52 ± 4.83^{c}	86.30 ± 3.90^{d}	7.15 ± 0.45^{d}	8.03 ± 0.47^{d}
7. Diabetic + glibenclamide	115.40 ± 8.65^{c}	96.00 ± 6.70^{d}	6.80 ± 0.32^{d}	7.50 ± 0.34^{d}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

Table 2: Changes in the level of tissue cholesterol and triglycerides in normal and experimental rats

Group	Cholesterol (mg/100 g wet tissue)			Triglycerides (mg/100 g wet tissue)		
	Liver	Kidney	Brain	Liver	Kidney	Brain
1. Normal	330.00 ± 18.12^{a}	436.50 ± 16.50^{a}	1425.50 ± 82.30^{a}	303.80 ± 12.30^{a}	459.10 ± 29.00^{a}	342.80 ± 22.30^{a}
2. Normal + CLEt	304.75 ± 13.10^{a}	407.70 ± 11.00^{a}	1307.30 ± 75.80^{b}	281.90 ± 11.50^{a}	423.60 ± 22.30^{a}	309.40 ± 21.10^{a}
3. Diabetic control	432.50 ± 29.0^{b}	692.30 ± 18.80^{b}	2633.80 ± 124.7^{c}	490.10 ± 20.60^{b}	609.40 ± 32.80^{b}	488.60 ± 27.60^{b}
4. Diabetic + CLEt (200 mg/kg)	345.90 ± 15.4^{c}	485.20 ± 22.0^{c}	1688.90 ± 104.1^{d}	362.40 ± 19.00^{c}	485.20 ± 30.80^{c}	365.00 ± 23.60^{c}
5. Diabetic + glibenclamide	351.7 ± 17.1^{d}	504.60 ± 16.9^{d}	1751.80 ± 102.7^{e}	374.20 ± 19.00^{c}	497.10 ± 30.30^{c}	378.90 ± 24.60^{c}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

level of tissue lipids, palmitic acid, stearic acid and oleic acid whereas the concentration of linolenic acid and arachidonic acid were increased when compared to diabetic control. Oral administration of CLEt to normal rats did not show any significant alterations on tissue lipids and fatty acid composition.

2.3. Discussion

Free radical mechanisms are involved in various tissue damages during diabetes [9]. In the present study, streptozotocin-induced diabetes mellitus characterized by hyperglycemia caused a significant rise in tissue lipid levels. The observed increase in the level of cholesterol in livers

Table 3: Changes in the level of tissue free fatty acids and phospholipids in normal and experimental rats

Group	Free fatty acids (mg/100 g wet tissue)			Phospholipids (mg/100 g wet tissue)		
	Liver	Kidney	Brain	Liver	Kidney	Brain
1. Normal	765.60 ± 27.3^{a}	814.10 ± 37.90^{a}	15.20 ± 2.05^{a}	1226.70 ± 63.9^{a}	1121.10 ± 74.30^{a}	2287.60 ± 112.10^{a}
2. Normal + CLEt	721.50 ± 23.8^{b}	770.40 ± 17.95^{b}	12.82 ± 1.14^{a}	1134.30 ± 58.5^{b}	1033.30 ± 56.47^{a}	2143.20 ± 82.20^{a}
3. Diabetic control	1253.00 ± 65.5^{c}	1654.80 ± 65.00^{c}	30.70 ± 2.98^{b}	2540.10 ± 79.2^{c}	2218.20 ± 94.60^{b}	2976.70 ± 119.20^{b}
4. Diabetic + CLEt	788.20 ± 28.9^{d}	846.80 ± 38.90^{d}	17.20 ± 2.11^{c}	1264.60 ± 45.4^{d}	1163.60 ± 77.60^{c}	2361.30 ± 119.20^{c}
5. Diabetic + glibenclamide	798.20 ± 34.5^{d}	866.10 ± 40.4^{d}	18.90 ± 2.21^{c}	1291.20 ± 41.5^{d}	1178.00 ± 77.40^{c}	2483.90 ± 98.10^{d}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

Table 4: Changes in the fatty acid composition of total liver lipids in normal and experimental rats

Group	Percentage of fatty acid/100 mg tissue				
	16 : 0 Palmitic acid	18 : 0 Stearic acid	18 : 1 Oleic acid	18 : 3 Linolenic acid	20 : 4 Arachidonic acid
1. Normal	19.57 ± 0.87^{a}	10.43 ± 1.22^{a}	7.42 ± 0.31^{a}	6.79 ± 0.34^{a}	20.66 ± 1.04^{a}
2. Normal + CLEt	17.93 ± 0.88^{b}	9.02 ± 0.32^{b}	6.97 ± 0.26^{a}	7.19 ± 0.35^{a}	21.36 ± 1.15^{a}
3. Diabetic control	26.43 ± 1.42^{c}	16.04 ± 1.20^{c}	12.39 ± 0.93^{b}	2.13 ± 0.10^{b}	13.25 ± 0.47^{b}
4. Diabetic + CLEt	21.13 ± 1.34^{d}	11.90 ± 0.93^{d}	9.31 ± 0.34^{c}	5.20 ± 0.22^{c}	18.25 ± 0.69^{c}
5. Diabetic + glibenclamide	24.88 ± 0.91^{d}	13.52 ± 0.48^{d}	10.10 ± 0.38^{c}	3.89 ± 0.15^{d}	17.11 ± 0.40^{d}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

Table 5: Changes in the fatty acid composition of total kidney lipids in normal and experimental rats

Group	Percentage of fatty acid/100 mg tissue				
	16 : 0 Palmitic acid	18 : 0 Stearic acid	18 : 1 Oleic acid	18 : 3 Linolenic acid	20 : 4 Arachidonic acid
1. Normal	21.09 ± 0.90^{a}	12.99 ± 0.72^{a}	5.42 ± 0.32^{a}	5.45 ± 0.27^{a}	13.00 ± 0.98^{a}
2. Normal + CLEt	20.16 ± 0.45^{a}	11.21 ± 0.50^{a}	4.45 ± 0.16^{a}	5.60 ± 0.21^{a}	13.41 ± 1.11^{a}
3. Diabetic control	30.73 ± 1.37^{b}	21.50 ± 1.28^{b}	10.21 ± 0.65^{b}	1.29 ± 0.09^{b}	5.81 ± 0.20^{b}
4. Diabetic + CLEt	23.68 ± 0.93^{c}	14.60 ± 0.79^{c}	6.73 ± 0.32^{c}	4.08 ± 0.24^{c}	11.20 ± 0.60^{c}
5. Diabetic + glibenclamide	26.10 ± 1.58^{c}	15.66 ± 0.95^{c}	8.98 ± 0.45^{d}	3.30 ± 0.14^{d}	10.00 ± 0.40^{d}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

Table 6: Changes in the fatty acid composition of total brain lipids in normal and experimental rats

Group	Percentage of fatty acid/100 mg tissue				
	16 : 0 Palmitic acid	18 : 0 Stearic acid	18 : 1 Oleic acid	18 : 3 Linolenic acid	20 : 4 Arachidonic acid
1. Normal	24.51 ± 2.20^{a}	12.98 ± 1.02^{a}	9.00 ± 0.40^{a}	8.61 ± 0.30^{a}	16.18 ± 1.28^{a}
2. Normal + CLEt	22.58 ± 1.23^{a}	11.96 ± 1.00^{a}	8.08 ± 0.52^{a}	8.02 ± 0.39^{a}	16.45 ± 1.30^{a}
3. Diabetic control	31.70 ± 2.44^{b}	20.28 ± 1.59^{b}	15.00 ± 1.11^{b}	3.52 ± 0.20^{b}	8.80 ± 0.40^{b}
4. Diabetic + CLEt	26.08 ± 1.78^{c}	14.38 ± 1.08^{c}	10.20 ± 0.62^{c}	6.51 ± 0.29^{c}	14.70 ± 1.00^{c}
5. Diabetic + glibenclamide	28.00 ± 1.62^{c}	16.48 ± 1.38^{d}	12.18 ± 0.93^{d}	5.39 ± 0.15^{c}	13.55 ± 0.75^{c}

Values are given as mean ± S.D. from ten rats in each group
Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT)
Duncan Procedure; Ranges for the level: 2.89; 3.04; 3.14; 3.20; 3.25

of streptozotocin diabetic rats could be due to increased cholesterogenesis since the activity of HMG-CoA reductase was reported to increase significantly in experimental diabetes [10].

The increased concentrations of free fatty acids in liver, kidney and brain may be due to lipid breakdown and this may cause increased generation of NADPH, which results in the activation of NADPH-dependent microsomal lipid peroxidation.

The significant increase in the level of triglycerides in liver, kidney and brain of diabetic rats may be due to the lack of insulin, since under normal conditions, insulin activates the enzyme, lipoprotein lipase, which hydrolyses triglycerides [11, 12]. Oral administration of CLEt to diabetic rats reversed all the above changes towards normal.

In our study there was a marked change in the fatty acid composition of total lipids in the liver, kidney and brain tissues. There was an increase in palmitic acid (16 : 0) and stearic acid (18 : 0) in the tissues of diabetic rats. This is in accordance with a previous report, which showed that there is a preferential synthesis of stearic acid and total saturated fatty acids in type 1 diabetic patients [13].

In our study we have also observed a significant increase in the concentration of oleic acid in diabetic rats. This observation correlated with an earlier study that showed an increased concentration of oleic acid in the membrane of both type 1 and type 2 diabetic patients [3].

In our study we have also observed a significant decrease in linolenic acid and arachidonic acid in diabetic rat tissues. Since these are rich in polyunsaturated fatty acids, they are the major targets for reactive oxygen species (ROS) damage. Diabetes reduced the rate-limiting desaturation steps, particularly delta-6 desaturation that converts linoleic acid to γ-linolenic acid and α-linolenic acid to stearidonic acid. Thus, the reduced availability of essential fatty acid intermediates in diabetes is further exacerbated by increased destruction due to elevated ROS [1].

Earlier reports have also shown that arachidonic acid is present in higher concentration in inflammed tissues than in healthy tissue [14].

Administration of CLEt resulted in a significant protection against the changes in the fatty acid composition and lipids in diabetic rats. This may be due to an improved glycaemic control and increased plasma insulin, which allows the diabetic rats treated with CLEt to maintain the tissue fatty acid composition at normal levels.

In conclusion, the present investigation shows that administration of CLEt to streptozotocin diabetic rats decreases tissue lipids and maintains the fatty acid composition in a normal range.

3. Experimental

3.1. Animals

Male albino Wistar rats of body weight 170–200 g bred in the Central Animal House, Rajan Muthiah Medical College, were used in this study. The animals were fed *ad libitum* with normal laboratoy pellet diet (Hindustan Lever Ltd., Mumbai, India) and water. The ethical committee. Annamalai University, approved the use of animals in the present study.

3.2. Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St. Louis, Mo, USA. The chemicals were of analytical grade.

3.3. Plant material

Coccinia indica leaves were collected freshly from the adjacent areas of Annamalai University. The plant was identified at the Herbarium of Botany Directorate in Annamalai University. A voucher specimen (No. 2438) was deposited in the Botany Department of Annamali University.

3.4. Preparation of plant extract

Fresh leaves of *Coccinia indica* (500 g) were chopped into small pieces, soaked overnight in 1.5 L of 95% ethanol. This suspension was filtered and the residue was resuspended in an equal volume of 95% ethanol for 48 h and filtered again. The two filtrates were pooled and the solvents

were evaporated in a rotavapor at 40–50 °C under redeuced pressure [6]. A dark semisolid (greenish-black) material was obtained (20–30 g). It was stored at 0–4 °C until used. When needed, the residual extract was suspendent in distilled water and used in the study [6].

3.5. Induction of experimental diabetes

A freshly prepared solution of streptozotocin (45 mg/kg) in 0.1 M citrate buffer, pH 4.5 was injected intraperitoneally in a volume of 1 ml/kg [15]. 48 h after streptozotocin administration, rats with moderate diabetes having glycosuria and hyperglycemia (i.e. with blood glucose of 200–300 mg/dl) were taken for the experiment.

3.6. Experimental design

In the experiment a total of 70 rats (50 surviving diabetic rats, 20 normal rats) were used. The rats were divided into 7 groups of 10 rats each. Group 1: Normal rats. Group 2: Normal rats given *Coccinia indica* Leaf Extract (CLEt) (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days [6–8]. Group 3: Diabetic control rats. Group 4: Diabetic rats given *Coccinia indica* Leaf Extract (CLEt) (50 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 5: Diabetic rats given CLEt (100 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 6: Diabetic rats given CLEt (200 mg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days. Group 7: Diabetic rats given Glibenclamide (600 µg/kg body weight) in aqueous solution daily using an intragastric tube for 45 days [16].
After 30 and 45 days, the animals were deprived of food overnight and blood was collected through sino-ocular puncture in a tube containing potassium oxalate and sodium fluoride for the estimation of blood glucose and plasma insulin. After the estimation of blood glucose and plasma insulin at day 45, the animals were sacrificed by decapitation. Liver, kidney and brain were dissected, patted dry and weighed.

3.7. Analytical methods

Fasting blood glucose was estimated by the O-toluidine method [17]. Plasma insulin was estimated by an enzyme linked immunosorbant assay (ELISA) kit (Boehringer Mannheim, Germany).
Liver, kidney and brain for lipids and fatty acid analysis were dried between folds of filter paper, weighed, and transferred to 2 : 1 chloroform–methanol (v/v) for extraction of lipids according to the procedure of Folch et al. [18]. Total Cholesterol was estimated by the method of Zlatkis et al. and triglycerides by the method of Foster and Dunn [19, 20]. Phospholipids and free fatty acids were estimated by the methods of Zilversmit and Davis and Falholt et al. [21, 22]. Fatty acid composition was determined according to the method of Morrision and Smith [23].

3.8. Statistical analysis

Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) [24].

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