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Hypolipidaemic effect of *Casearia esculenta* root extracts in streptozotocin-induced diabetic rats

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Received November 4, 2003, accepted April 30, 2003

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Pharmazie 58: 828–832 (2003)

The hypolipidaemic effect of an aqueous extract of *Casearia esculenta* root, an indigenous antidiabetic medicine popularly used in rural South India was investigated. Administration of the extract of *C. esculenta* (200 and 300 mg/kg body wt.) for 45 days resulted in significant reduction in serum and tissue cholesterol, phospholipids, free fatty acids and triglycerides in streptozotocin (STZ) diabetic rats. In addition to that, significant ($p < 0.05$) decrease in high density lipoprotein (HDL) whereas significant increase ($p < 0.05$) in low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were observed in STZ diabetic rats, which was normalized after 45 days of *C. esculenta* root extract treatment. The root extract at dose of 300 mg/kg body wt. showed much significant hypolipidaemic effects than the dose of 200 mg/kg body weight.

1. Introduction

Diabetes mellitus (DM) is characterised by persistent elevation of blood glucose above the normal range due to the absolute or relative lack of insulin, leading to abnormalities of carbohydrates, lipid and protein metabolism [1]. Lipid disorders are common in both insulin dependent and non-insulin dependent diabetes mellitus and are related to the degree of glycemic control [2].

Hyperlipidaemias are major modifiable risk factor for the initiation and progression of cardiovascular disease [3–8]. Because of the putative link between abnormal lipid metabolisms and incidence of diabetes mellitus, a great deal of effort has gone into the identification of chemical entities that can lower the lipid content of the blood. The currently available hypolipidaemic agents lack the desired properties of an ideal drug and often result in patient noncompliance. Therefore researchers are involved to find an effective, safe and less expensive drug. Several plant materials are being investigated for this purpose [9, 10].

Casearia esculenta Roxb. (Flacourtiaceae) is such a plant popularly known as "Kadala-Zhinjill", "Kottarkovai" in Tamil "Wild cowrie fruit" in English and "Saptarangi" in Sanskrit. It is a shrub richly distributed in Konkan plateu, South India. In Indian traditional medicine, the plant has been a popular remedy for the treatment of diabetes mellitus [11–13] and is one of the major ingredients of D-400, a large selling antidiabetic drug in India (Himalaya drug Co, Bangalore) [14]. In our previous communication we reported the glycemic control by *C. esculenta* – a short duration study in albino rats [15] and antihyperglycemic property of *C. esculenta* root extract on normal and streptozotocin diabetic rats [16]. To our knowledge, no detailed investigations have been carried out to shed light on the hypolipidaemic property of *C. esculenta* root extract. Thus, the effect of *C. esculenta* root extract (CEEt) on plasma and tissue lipid parameters in STZ diabetic rats was studied.

2. Investigations, results and discussion

Table 1 shows the record of body and organ weight changes in normal and streptozotocin diabetic rats. Administration of *C. esculenta* (200 and 300 mg/kg body weight) and glibenclamide exhibits a remarkable improvement in liver and body weight and significant reduction in kidney weight as compared to untreated diabetic rats. *C. esculenta* at dose of 300 mg/kg body weight is more effective than 200 mg body weight.

Table 2 demonstrates the serum levels of total cholesterol, phospholipids, free fatty acids and triglycerides, in control and experimental animals. A significant elevation was observed in serum lipid parameters in diabetic rats when compared with control rats. Oral administration of *C. esculenta* (200 and 300 mg/kg body weight) and glibenclamide significantly decreased the levels of lipid parameters as compared to untreated diabetic rats.

Table 3 shows the serum levels of lipoproteins (high density lipoproteins (HDL-C), low density lipoprotein (LDL-C) and very low density lipoprotein (VLDL-C)) in control and experimental animals. A significant elevation of serum LDL and VLDL and significant reduction in HDL-C is observed in diabetic rats when compared with control rats. Oral administration of *C. esculenta* (200 and 300 mg/kg body weight) and glibenclamide significantly decreases the levels of LDL and VLDL while significant improvement in HDL was observed as compared to untreated diabetic rats.

Tables 4 and 5 show the tissue concentration of cholesterol, phospholipid, tryglycerides and free fatty acids in

Table 1: Effect of *C. esculenta* root extract on body weight and tissue weight (liver and kidney) changes in normal and STZ diabetic rats

Group	Treatment (dose/kg body wt.)	Body weight (g)		Liver weight (g)	Kidney weight (g)
		Initial	Final		
I	Control	162.5 ± 2.73	202.5 ± 6.8*	6.60 ± 0.21[a]	1.06 ± 0.03[a]
II	Diabetic control	165.83 ± 3.76	150.83 ± 3.76[b]	4.28 ± 0.14[b]	1.50 ± 0.07[b]
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	160.80 ± 2.00	162.50 ± 2.60[c]	5.70 ± 0.21[c]	1.28 ± 0.05[c]
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	159.16 ± 2.10	163.33 ± 2.60[c]	5.93 ± 0.15 [d]	1.08 ± 0.05[a]
V	Diabetic + glibenclamide (600 µg/kg body wt.)	161.66 ± 2.58	167.50 ± 5.24[c]	6.13 ± 0.07[d]	1.12 ± 0.07[a]

Values are given as mean ±SD for six animals in each group
Values not sharing a common superscripts differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 2: Effect of *C. esculenta* root extract on serum cholesterol, free fatty acids, phospholipids and triglycerides of control and experimental animals

Group	Treatment (dose/kg body wt)	Cholesterol (mg/dL)	Free fatty acids (mg/dL)	Phospholipids (mg/dL)	Triglycerides (mg/dL)
I	Control (2% gum acacia)	63.32 ± 4.69[a]	50.26 ± 2.26[a]	125.0 ± 7.81[a]	50.75 ± 2.71[a]
II	Diabetic control	229.00 ± 17[b]	135.46 ± 2.56[b]	218.75 ± 13.11[b]	157.16 ± 4.42[b]
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	134.08 ± 6.89[c]	86.42 ± 1.43[c]	189.58 ± 18.39[c]	120.25 ± 7.90[c]
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	111.36 ± 6.89[d]	62.14 ± 1.95[d]	160.41 ± 12.28[d]	82.00 ± 5.75[d]
V	Diabetic + glibenclamide (600 µg/kg body wt.)	97.72 ± 6.89[d]	60.6 ± 1.43[d]	139.58 ± 9.40[a]	76.6 ± 4.69[d]

Values are given as mean ±SD for six animals in each group
Values not sharing a common superscripts differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

liver and kidney of normal and experimental animals. The levels of tissue lipid parameters significantly ($p < 0.05$) increase in diabetic rats as compared to normal rats. Administration of *C. esculenta* (200 and 300 mg/kg body weight), and glibenclamide decreases tissue cholesterol, phospholipid, triglycerides and free fatty acids significantly as compared to diabetic rats. For all the parameters studied, *C. esculenta* (300 mg/kg body weight) shows a significant lipid lowering effect when compared with *C. esculenta* (200 mg/kg body weight).

Streptozotocin (STZ), a nitroso derivative of D-glucosamine has been used to induce a "chemical diabetes (STZ diabetes) in a wide variety of animal species through the partial destruction of insulin secreting β-cells [17]. Induction of diabetes in these animals was confirmed as a significant rise in blood glucose and elevated tissue lipids.

The present study demonstrated that the *C. esculenta* root extract protects diabetic rats from massive body weight loss, when given orally, daily for 45 days, compared with untreated diabetic rats. The ability of the plant extract to protect body weight loss seems to be due to its ability to reduce the level of serum lipids, secondary to its hypoglycemic effect. A decrease in the liver weight is observed in diabetic animals might be due to an increased breakdown of glycogen and/or pronounced gluconeogenesis. After 45 days of *C. esculenta* treatment in diabetic animals the liver weight increased due to the accumulation of glycogen and suppression of gluconeogenesis by *C. esculenta*.

In the present study we also observed an increased whole kidney weight in diabetic animals when compared with normal rats. Seyer and Hansen [18] and Osterby and Gundersen [19] reported a 15% increment of whole kidney weight within 72 h of induction of streptozotocin in experimental diabetic rats. This is due to the reason for the glomerular enlargement and accompanying glomerular cell proliferation in the early phase of streptozotocin-induced diabetic rats. In our present study, oral administration of *C. esculenta* significantly decreased the kidney weight to near normal values. This may be due to antihyperglycemic

Table 3: Effect of *C. esculenta* root extract on plasma lipoproteins (HDL-C, LDL-C and VLDL-C) and serum total lipids of control and experimental animals

Group	Treatment (dose/kg body wt)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	Serum total lipids (mg/dL)
I	Control (2% gum acacia)	30.55 ± 2.78[a]	41.95 ± 5.54[a]	10.97 ± 0.96[a]	244.65 ± 10.57[a]
II	Diabetic control	17.85 ± 1.30[b]	192.81 ± 10.66[b]	18.34 ± 0.76[b]	669.21 ± 31.93[b]
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	23.40 ± 1.79[c]	96.97 ± 8.15[c]	14.23 ± 1.33[c]	375.97 ± 14.95[c]
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	27.37 ± 1.99[d]	72.64 ± 6.21[d]	11.56 ± 0.80[a]	294.31 ± 26.23[d]
V	Diabetic + glibenclamide (600 µg/kg body wt.)	29.74 ± 1.19[d]	56.60 ± 7.88[c]	11.46 ± 0.49[a]	280.30 ± 14.68[a]

Values are given as mean ±SD for six animals in each group
Values not sharing a common superscripts differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 4: Effect of *C. esculenta* root extract on liver cholesterol, free fatty acids, phospholipids and triglycerides of control and experimental animals

Group	Treatment (dose/kg body wt)	Cholesterol (mg/g wet tissue)	Free fatty acid (mg/g wet tissue)	Phospholipids (mg/g wet tissue)	Triglycerides (mg/g wet tissue)
I	Control (2% gum acacia)	3.2 ± 0.28^a	7.93 ± 0.92^a	21.04 ± 2.55^a	16.94 ± 1.05^a
II	Diabetic control	7.86 ± 0.68^b	18.86 ± 2.93^b	54.16 ± 5.40^b	38.75 ± 1.62^b
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	5.53 ± 0.46^c	12.12 ± 1.63^c	40.41 ± 1.88^c	23.39 ± 1.97^c
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	4.2 ± 0.61^d	9.97 ± 1.64^d	26.30 ± 2.75^d	20.44 ± 0.76^d
V	Diabetic + glibenclamide (600 μg/kg body wt.)	3.66 ± 0.58^a	8.48 ± 1.13^a	22.08 ± 3.67^a	18.06 ± 2.48^a

Values are given as mean ±SD for six animals in each group
Values not sharing a common superscripts differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 5: Effect of *C. esculenta* root extract on kidney cholesterol, free fatty acids, phospholipids and triglycerides of control and experimental animals

Group	Treatment (dose/kg body wt)	Cholesterol (mg/g wet tissue)	Free fatty acid (mg/g wet tissue)	Phospholipids (mg/g wet tissue)	Triglycerides (mg/g wet tissue)
I	Control (2% gum acacia)	5.46 ± 0.60^a	5.44 ± 0.87^a	13.75 ± 1.20^a	5.24 ± 0.49^a
II	Diabetic control	10.53 ± 0.78^b	18.13 ± 1.53^b	30.41 ± 3.67^b	13.77 ± 0.71^b
III	Diabetic + *C. esculenta* (200 mg/kg body wt.)	6.73 ± 0.46^a	8.61 ± 0.80^c	22.50 ± 2.47^c	9.83 ± 0.72^c
IV	Diabetic + *C. esculenta* (300 mg/kg body wt.)	5.4 ± 0.55^a	7.48 ± 0.86^d	18.75 ± 1.62^d	6.77 ± 0.45^a
V	Diabetic + glibenclamide (600 μg/kg body wt.)	5.73 ± 0.65^a	6.00 ± 0.74^a	14.79 ± 1.00^a	6.88 ± 0.75^a

Values are given as mean ± SD for six animals in each group
Values not sharing a common superscripts differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT

effect of *C. esculenta*, which reduces the diabetes-induced abnormalities in kidney.

The levels of serum lipids are usually elevated in diabetes mellitus, and such an elevation represents a risk factor for coronary heart disease [20]. Lowering of serum and tissue lipids concentration through diet or drugs seems to be associated with a decrease in the risk of vascular disease [21]. The abnormal high concentration of serum lipids in the diabetic subjects is mainly, due to the increase in the mobilization of free fatty acids from the peripheral fat depots [22].

Phospholipids are vital components of biomembranes and play an important role in the transport of triglycerides [23, 24]. Any disease process that interferes with the synthesis of phospholipids will impede the transport of triglycerides from liver and will therefore lead to fatty liver [25].

Metabolilc aberration in alloxan diabetic rats suggests a high turnover of triglycerides and phospholipids [26]. *C. esculenta* may antagonise the metabolic aberration and thereby restore the normal metabolism by tilting the balance from high lipids to high carbohydrate turnover.

The primary pathology of the diabetic kidney involves a marked thickening of the glomerulous basement membrane [27]. Changes in fatty acid during diabetes are closely associated with changes in the activity of Na^+/K^+ATPase in the kidney [28]. Accumulation of fatty acids results in higher levels of their metabolites such as acyl carnitine and long chain acylCoA, which interferes with Na^+/K^+ATPase activity [29], impairing the active transport of Na^+ and K^+ ions. This defect may be implicated in diabetic nephropathy. This hypothesis is supported by the observation of Kumthekar and Katyare [30] reporting that Na^+/K^+ATPase activity decreased in the streptozotocin diabetic rat kidney. Thus, the diabetic complications associated with renal tissue may be partly due to abnormalities in lipid metabolism.

High level of total cholesterol and, more importantly, LDL cholesterol are major coronary risk factors [8]. Oral administration of *C. esculenta* root extract for 45 days resulted in a significant lowering of both total and LDL cholesterol. Several studies show that an increase in HDL cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also decrease HDL cholesterol [31–33]. In the present study we observed a significant lowering of LDL and a significant improvement in HDL cholesterol. This is an important advantage in treatment of hypercholesterolaemia, especially among Indians where low HDL cholesterol is the most prevalent lipoprotein abnormality [34]. Total cholesterol/HDL and LDL/HDL cholesterol ratio are also predictor of coronary risk [8]. In the present study rats treated with *C. esculenta* had markedly reduce ratios. One of the possible actions of *C. esculenta* may be due to its inhibition of endogenous synthesis of cholesterol and enhancement of the degradation of formed cholesterol by increasing the excretion through intestinal tract.

Enhanced hexokinase activities in *C. esculenta* treated rats suggests a greater uptake of glucose from the body into the liver cells. Enhanced activities of these enzymes suggest that enhanced lipid metabolism during diabetes is shifted toward carbohydrate metabolism and it enhances the utilization of glucose at the peripheral sites [16].

The ability of *C. esculenta* to reduce the levels of serum and tissue lipids in diabetic subjects has never been studied before. The result of this study revealed that a continuous administration of *C. esculenta* extract for 45 days prevents elevation of serum and tissue lipids secondary to

the diabetic state. The hypolipidaemic effect of *C. esculenta* could be explained as a direct result of the reduction in the blood glucose concentration.

3. Experimental

3.1. Plant material

Root of *Casearia esculenta* was collected from Western ghats of Tamil Nadu and the plant was authenticated by Dr. C. Chelladurai, Research Officer, Survey Medicinal Plant Unit (S.M.P.U.), Central Council for Research in Siddha and Ayurvedic, Siddha Medical College, Palayamkottai, Tamilnadu. A voucher specimen was deposited in the (AU2145) Department of Botany, Annamalai University, Annamalainagar, Tamilnadu. The plant root was air dried at 25 °C in the room and the dried root was made into fine powder with auto-mix blender and the powdered part was kept in deep freezer until the time of use.

3.2. Preparation of aqueous extract

100 g of dry fine powder was suspended in 250 ml water for 2 h and then boiled at 60–65 °C for 30 min (since boiled decoction of root of this plant has been used as remedy for diabetes). The extract was preserved and the processes were repeated for three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through a fine cotton cloth. The filtrate upon evaporation at 40 °C yielded 12% semi-solid extract.

3.3. Drugs and chemicals

Streptozotocin (STZ) was obtained form Sigma chemical company. All other chemicals used were of analytical grade.

3.4. Animals

Male Wistar albino rats (weighing 140–160 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College, Annamalai University, Annamalai Nagar. Animals were maintained at Central Animal House and the animals were fed on standard diet (Hindustan Lever, Bangalore) and water *ad libitum*. All studies were conducted in accordance with the National Institute Health "Guide for the Care and Use of Laboratory Animals" [35] and the study was approved by the Ethical Committee of Rajah Muthaih Medical College and Hospital, Annamalai University, Annamalainagar.

3.5. Experimental induction of diabetes

Adult (9 weeks old) male Wistar rats were made diabetic with an intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight) dissolved in citrate buffer (0.1 M, pH 4.5). Streptozotocin injected animals exhibited massive glycosuria and hyperglycemia within a few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, 96 h after injection with STZ. Albino rats with blood glucose level above 240 mg/dl were considered to be diabetic and were used in the experiment. Six rats receiving 2% gum acacia alone served as control.

3.6. Treatment groups

After the induction of diabetes the rats were divided into five groups of six animals each.
Group I: Control rats received vehicle solution (2% gum acacia). Group II: Diabetic control. Group III: Diabetic rats received *Casearia esculenta* root extract (200 mg/kg body weight) in 2% gum acacia using an intragastric tube daily for 45 days. Group IV: Diabetic rats given *C. esculenta* root extract (300 mg/ kg body weight) in 2% gum acacia using an intragastric tube daily for 45 days. Group V: Diabetic rats received glibenclamide orally (600 μg/kg body weight) as aqueous solution using an intragastric tube daily for 45 days.
After 45 days of treatment, the animals were sacrificed by cervical decapitation. The blood was collected in heparinised centrifuge tubes and the plasma were collected for the estimations of lipid profile. The liver and kidney was dissected out immediately and washed with ice cold saline. A portion of the tissue was homogenized using a potter Elvejham homogenizer and lipid extraction was prepared according to the method of Folch et al. [36], which is further used for the estimations of cholesterol, phospholipid, triglycerides and free fatty acids.

3.7. Biochemical analysis

3.7.1. Free fatty acids

Free fatty acids (FFA) were estimated by the method of Falholt et al. [37]. An aliquot of the lipid extract was evaporated and treated with 6 ml of chloroform-heptane-methanol solvent (5 : 5 : 1). 200 mg of activated silicic acid was added, and the mixture left aside for 30 min and centrifuged. The supernatant was transferred to tubes containing 2.5 ml of copper-triethanolamine reagent, mixed in a mechanical stirrer and centrifuged at 1000 g for 20 min. 3 ml of the upper layer was treated with 0.5 ml of diphenyl carbazide reagent and the colour developed read at 540 nm.

3.7.2. Phospholipid

Phospholipids were estimated by the method of Zilversmit and Davis [38]. The organic phosphorus in the lipid was converted to inorganic phosphorus by digesting with 1 ml of concentrated nitric acid and 1 ml of 5 N sulfuric acid to a colourless solution. 1 ml of water was added, and the mixture boiled for 15 s, followed by the addition of 1 ml ammonium molybdate and 0.4 ml 1-amino-2-napthol-4-sulfonic acid. The colour developed was read at 680 nm.

3.7.3. Triglycerides

Triglycerides (TG) were estimated by the method of Foster and Dunn [39]. To an aliquot of dried lipid extract, 4 ml isopropanol and 400 mg of alumina were added, shaken well for 15 min and centrifuged at $1{,}000 \times g$ for 10 min. A saponifying agent (5 g potassium hydroxide in 60 ml distilled water and 40 ml isopropanol) was added to the supernatant and incubated at 65 °C for 15 min. Then sodium metaperiodate and acetyl acetone reagents were added, mixed and the tubes were again incubated at 65 °C for 30 min. The colour developed was read at 420 nm.

3.7.4. Cholesterol and lipoprotein

Serum total cholesterol and HDL-cholesterol was analysed in an autoanalyser using the reagent kit obtained from Boehringer Mannhein, Germany. LDL and VLDL cholesterol was calculated as follows [40].

$$\text{LDL} = \text{Total cholesterol} - (\text{HDL} + \text{VLDL})$$

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

Serum total lipid content was calculated as recommended by Philips et al. [41]. Total serum lipid (mg/dL) = 2.27 × serum cholesterol (mg/dL) + serum triglycerides (mg/dL) + 0.623.

3.8. Statistical analysis

Values are presented as mean ±S.D., data were analysed using Analysis of Variance (ANOVA) and group mean were compared with Duncan's multiple range test (DMRT).

Acknowledgement: Authors are thankful to Indian Council of Medical Research (ICMR), New Delhi for providing Senior Research Fellow (SRF) to one of the authors (A.P).

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