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Glycaemic control by *Casearia esculenta* – a short duration study in albino rats

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An aqueous extract of *Casearia esculenta* was found to lower blood glucose in basal conditions and after a glucose load in normal rats. Maximum reduction in blood glucose was observed between 2–3 h at a dose level of 200 and 300 mg/kg body weight. *C. esculenta* extract was also found to reduce the blood sugar level in streptozotocin – induced diabetic rats. Oral administration of the extract significantly reduced the blood sugar in streptozotocin induced diabetic rats for 15 days. The extract was also found to reduce the increased plasma thiobarbituric acid reactive substances (TBARS), blood urea and improvement in body weight reduction induced by streptozotocin injection. These results indicate that *C. esculenta* extracts are able to ameliorate biochemical changes induced by streptozotocin in diabetic rats.

1. Introduction

Traditional medicinal plants are used throughout the world for a range of diabetic conditions [1, 2]. The growing trend in third world countries towards natural medicines has stimulated a new wave of research in traditional practices.

In rural South India, decoction of *Casearia esculenta* roots has been popularly used in the folklore treatment of diabetes. *Casearia esculenta* Roxb. (Flacourtiaceae) popularly known as "Kadala-Zhinjill", "Kottarkovai" in Tamil "Wild cowrie fruit" in English and "Saptarangi" in Sanskrit is a shrub richly distributed in Konkan platea and South India. In Indian traditional medicine, the plant has been a popular remedy for the treatment of diabetes mellitus [3–5] and our study drug is one of the major ingredient of D-400, a largest selling antidiabetic drug in India (Himalaya drug Co, Bangalore) [6]. The first scientific study was undertaken by Gupta et al. [7] who reported the hypoglycemic effect of this plant in rats and rabbits. Then Choudhury and Basu [8] reported that *C. esculenta* root extract did contain uncharacterized hypoglycemic factor(s) which reduced blood sugar level. Preliminary results were highly encouraging revealing that blood glucose level was significantly lowered after oral administration of *C. esculenta* root extract in normal, glucose load and streptozotocin induced state and no harmful side effects were observed throughout the study. To our knowledge, no detailed investigations have been carried out to shed light on the anti-diabetic effect of *C. esculenta*. Thus, the present investigation should evaluate the anti-diabetic effect of *C. esculenta* root extract in normal and streptozotocin (STZ) diabetic rats.

2. Investigations and results

2.1. Estimation of blood glucose level and other investigations

Blood samples were collected through the tail vein just prior to and on days 4, 7, and 15 after the STZ injection. The following biochemical investigations were carried out in our laboratory (a) blood glucose by the o-toluidine method [9], OGTT [10] and blood urea by an auto analyzer using Boehringer Mannheim Kit. Body weight changes were recorded periodically.

Percent of glycemic changes was calculated as function of time, by applying the formula [11].

$$\%\ \text{glycemic changes} = \frac{\text{Gx} - \text{Go}}{\text{Go}} \times 100$$

Go = initial glycemia values
Gx = glycemia values at x minutes time interval

2.2. Estimation of lipid peroxidation products

Thiobarbituric acid was added to plasma samples under acidic conditions and the absorbance of colour that developed after heating was estimated spectrophotometrically at 535 nm [12]. 1,1′3,3′-tetramethoxy propane was used as an internal standard and the concentration was expressed in nmol of malondialdehyde (MDA) per ml of plasma.

2.3. Results

Administration of *C. esculenta* root extract was found to reduce blood glucose level in normal rats (Table 1). The maximum reduction in blood glucose was noted 3 h after the administration of the extract. The reduction was maximum at the 300 mg/kg body weight dose level.

The effect of *C. esculenta* extract on glucose tolerance is given in Table 2. In glucose fed rats (2 g/kg body weight) administration of 300 mg/kg body weight of *C. esculenta* significantly increased the tolerance for glucose, the maximum glucose tolerance was noted for test dose level at the 300 mg/kg body wt. after glucose loading (2 h after drug dosing).

The effect of *C. esculenta* root extract in streptozotocin induced diabetic rats is given in Table 3. The fasting blood glucose level in streptozotocin-diabetic rats was 240–260 mg/dl. The initial reduction in blood glucose was observed 2 h after the administration of *C. esculenta* extract. Chronic administration of *C. esculenta* root extract was found to significantly decrease the blood glucose levels (Table 4).

Plasma thiobarbituric acid reactive substances (TBARS) (an index of lipid peroxidation) was measured to be 1.86 nmol/ml in normal rats which was increased to 3.02 nmol/ml on day 15 of STZ rats. Administration of *C. esculenta* extract 300 mg/kg body weight significantly reduced plasma TBARS level to 2.22 and 2.09 nmol/ml on 7th and 15th day, respectively (Table 5).

The normal functioning of the kidney was assessed as blood urea level, which was remarkably elevated in dia-

Table 1: Effect of *C. esculenta* root extract on blood glucose level in fasted normal rats

Group	Treatment dose/kg body wt	Blood glucose (mg/dl)			
		Fasting	1 h	2 h	3 h
I	Control – received vehicle only	64.3 ± 3.7^{a}	64.3 ± 2.1^{a}	62.7 ± 3.3^{a}	64.3 ± 2.1^{b}
II	*C. esculenta* 100 mg	67.3 ± 3.8 ^{a}	65.1 ± 2.4^{b}	63.5 ± 2.4^{a}	62.7 ± 1.2^{b}
III	*C. esculenta* 200 mg	64.3 ± 5.7^{a}	59.5 ± 2.1^{a}	54.0 ± 2.4	47.7 ± 4.2^{a}
VI	*C. esculenta* 300 mg	69.0 ± 2.1^{a}	65.1 ± 2.4^{b}	60.3 ± 6.5^{a}	45.6 ± 6.5^{a}
V	*C. esculenta* 400 mg	65.4.4 ± 4.2^{a}	66.7 ± 0.1^{b}	63.5 ± 2.4^{a}	55.3 ± 2.4

Values are mean ± S.D for six animals in each group
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 2: Effect of *C. esculenta* root extract on oral glucose tolerance in rats (2 g/kg body weight)

Group	Treatment dose/kg body wt	Blood glucose (mg/dl)			
		Fasting	30 min	60 min	90 min
I	Control + glucose 2 g	69.0 ± 2.1^{a}	146 ± 8.9	130 ± 4.9	122.2 ± 9.4
II	*C. esculenta* 200 mg + glucose	69.8 ± 2.5^{a}	125.4 ± 5.0^{a}	114.3 ± 4.2^{a}	93.6 ± 5.0
III	*C. esculenta* 300 mg + glucose	64.3 ± 7.7^{a}	127.0 ± 2.4^{a}	114.3 ± 4.3^{a}	83.3 ± 2.1^{a}
VI	Glibenclamide 600 µg/kg body wt + glucose	65.1 ± 2.5^{a}	128.6 ± 4.3^{a}	103.2 ± 2.5^{a}	76.2 ± 4.3^{a}

Values are mean ± S.D for six animals in each group
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 3: Effect of *C. esculenta* root extract on blood glucose levels in streptozotocin diabetic rats

Group	Treatment dose/kg body wt	Blood glucose (mg/dl)			
		Fasting	1 h	2 h	3 h
I	Control (2% gum acacia)	66.6 ± 4.25	65.7 ± 3.0^{a} (−1.38)	64.8 ± 2.6^{a} (−2.85)	63.8 ± 3.0^{a} (−4.32)
II	Diabetic control	250.2 ± 8.2^{a}	247.1 ± 5.3^{bc} (−1.25)	241.2 ± 5.3^{bc} (−3.60)	239.2 ± 3.0^{b} (−4.39)
III	Diabetic + *C. esculenta* 200 mg	248.3 ± 8.8^{a}	241.2 ± 5.2^{b} (−2.87)	237.2 ± 3.0^{b} (−4.45)	233.3 ± 3.0^{c} (−6.02)
VI	Diabetic + *C. esculenta* 300 mg	253.9 ± 7.8^{a}	250.9 ±13.2^{bc} (−1.17)	241.1 ± 10.5^{bc} (−5.0)	224.4 ± 5.3^{cd} (−11.63)
V	Diabetic + glibenclamide 600 µg	258.7 ± 6.5^{a}	252.9 ± 10.5^{c} (−2.23)	239.2 ± 13.2^{c} (−7.54)	225.5 ± 8.0^{d} (−12.84)

Values are mean ± S.D for six animals in each group
Values in parenthesis indicate the percentage glycaemic changes
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 4: Effect of continuous administration (15 days) of *C. esculenta* extract on blood glucose level in streptozotocin treated rats

Group	Treatment dose/kg body wt	Blood glucose (mg/dl)			
		Initial day	Day 4	Day 7	Day 15
I	Control (2% gum acacia)	66.7 ± 4.3^{a}	68.6 ± 3.0 (+2.87)	69.6 ± 4.4 (+4.34)	71.5 ± 4.4 (+7.22)
II	Diabetic control	250.2 ± 8.2^{a}	269.9 ± 5.8 (+7.85)	277.9 ± 7.5 (+11.08)	288.9 ± 6.6 (+15.49)
III	Diabetic + *C. esculenta* 200 mg	248.3 ± 8.8^{a}	242.9 ± 2.7^{a} (−2.19)	230.1 ± 3.8 ^{a} (−7.31)	217.6 ± 3.1^{a} (−12.34)
VI	Diabetic + *C. esculenta* 300 mg	253.9 ± 7.8^{a}	236.8 ± 6.1^{a} (−6.77)	224.9 ± 6.1^{a} (−11.40)	208.8 ± 3.1^{ab} (−17.77)
V	Diabetic + glibenclamide 600 µg	258.7 ± 6.5^{a}	238.2 ± 5.4^{a} (−7.91)	214.7 ± 4.4 (−17.00)	202.2 ± 5.4^{a} (−21.83)

Values are mean ± S.D for six animals in each group
Values in parenthesis indicate the percentage glycaemic changes
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 5: Effect of *C. esculenta* root extracts on plasma TBARS in streptozotocin diabetic rats

Group	Treatment dose/kg body wt	plasma TBARS (nmol/ml)			
		Initial day	Day 4	Day 7	Day 15
I	Control (2% gum acacia)	1.86 ± 0.2	1.77 ± 0.27	1.75 ± 0.13	1.42 ± 0.27
II	Diabetic control	2.30 ± 0.27[a]	2.48 ± 0.13[a]	2.84 ± 0.27	3.02 ± 0.27
III	Diabetic + *C. esculenta* 200 mg	2.48 ± 0.27[a]	2.30 ± 0.23[a]	2.22 ± 0.13[a]	2.13 ± 0.27[a]
VI	Diabetic + *C. esculenta* 300 mg	2.61 ± 0.18[a]	2.48 ± 0.27[a]	2.22 ± 0.13[a]	2.09 ± 0.29[a]
V	Diabetic + glibenclamide 600 μg	2.61 ± 0.18[a]	2.39 ± 0.23[a]	2.13 ± 0.24[a]	1.95 ± 0.36[a]

Values are mean ± S.D for six animals in each group
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 6: Effect of *C. esculenta* root extract on blood urea in streptozotocin diabetic rats

Group	Treatment dose/kg body wt	Blood urea (mg/ml)			
		Initial day	Day 4	Day 7	Day 15
I	Control (2% gum acacia)	18.2 ± 0.3	18.2 ± 0.3	18.5 ± 0.3	18.73 ± 0.4
II	Diabetic control	59.7 ± 2.8[a]	68.60 ± 1.7	77.9 ± 1.8	83.9 ± 3.1
III	Diabetic + *C. esculenta* 200 mg	56.9 ± 2.7[ab]	52.8 ± 2.1[a]	46.1 ± 1.6	38.6 ± 2.7
VI	Diabetic + *C. esculenta* 300 mg	57.7 ± 5.5[ab]	48.1 ± 1.3	36.8 ± 2.2[a]	33.0 ± 2.9
V	Diabetic + glibenclamide 600 μg	55.0 ± 1.2[a]	54.5 ± 1.9[a]	38.5 ± 1.8[a]	29.3 ± 2.8

Values are mean ± S.D for six animals in each group
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (DMRT)

Table 7: Effect of *C. esculenta* root extract on body weight in streptozotocin diabetic rats

Group	Treatment dose/kg body wt	Body weight (g)			
		Initial day	Day 4	Day 7	Day 15
I	Control (2% gum acacia)	162.5 ± 2.7[a]	163.3 ± 2.6[a]	170.7 ± 1.0	176.0 ± 5.5
II	Diabetic control	165.8 ± 4.5[a]	165.0 ± 4.5[a]	161.3 ± 1.0[a]	160.7 ± 1.0
III	Diabetic + *C. esculenta* 200 mg	161.0 ± 4.5[a]	163.0 ± 1.5[a]	163.3 ± 1.0[a]	166.7 ± 2.6[a]
VI	Diabetic + *C. esculenta* 300 mg	159.2 ± 2.0[a]	161.0 ± 0.8[a]	163.7 ± 1.4[a]	166.7 ± 1.4[a]
V	Diabetic + glibenclamide 600 μg	161.7 ± 2.6[a]	160.7 ± 1.03[a]	163.3 ± 2.5[a]	164.7 ± 0.5[a]

Values are mean ± S.D for six animals in each group
Values not sharing a common superscript differ significantly at $p < 0.05$ Duncan's Multiple Range Test (D)

betic animals when compared with control animals. Administration of *C. esculenta* extract 200 and 300 mg/kg body weight along with streptozotocin results in a significant fall of blood urea levels (Table 6).

A reduction in body weight was observed in STZ diabetic animals, but when the animals were treated with *C. esculenta* extract, the decrease in body weight was minimized to almost zero and even an improvement in body weight was observed (Table 7).

2.4. Statistical analysis

For statistical analysis, two-way analysis of variance (ANOVA) with interaction effects was employed. Inter group comparison were done using Duncan's Multiple Range Test (DMRT) (SPSS*).

3. Discussion

The aim of the present study was to evaluate the antidiabetic activity of *C. esculenta*, and to validate the use of this plant in diabetes mellitus control. Our results show that an aqueous extract of *C. esculenta* is able to reduce blood glucose levels in normal, glucose loaded and streptozotocin diabetic rats.

Oral administration of *C. esculenta* root extract significantly reduced blood glucose level in normal rats and the test drug also remarkably improved oral glucose tolerance in glucose-fed rats. At the present juncture, it is not possible to pinpoint the mechanism of antihyperglycaemic action of the extract of *C. esculenta*. However, based on an earlier report some suggestion can be made for its possible mechanism. It is reported that an infusion of *C. esculenta* blocks glucose absorption from gut [13] Thus, retardation of intestinal glucose absorption may also be partially responsible for inhibition of hyperglycaemia in glucose-fed rats. It has further been noted from earlier *in vitro* studies with rat hemidiaphram that prolonged treatment with the drug rendered the tissue more sensitive to insulin, the increased sensivity of the tissue to endogenous insulin is likely to lessen the requirement of the endogenous hormone and help in regeneration of damaged pancreatic islet cells [6]. Since the blood glucose lowering effect of the extract of *C. esculenta* was observed in both fasted normal and STZ diabetic rats, this effect could due to an increased peripheral glucose utilization.

STZ has been shown to generate lipid peroxidation and DNA strand breaks in pancreatic islet cells [14]. The pancreas in especially susceptible to the action of streptozotocin-induced free radical damage, administration of *C. esculenta* can reduce the level of serum lipid peroxides. These results confirm the possibility that a major function of the extract is protection of vital tissues including liver, kidney, brain and pancreas, thereby reducing the causation of diabetes (Table 5).

A reduction in body weight was observed in STZ-induced diabetic animals, but when the animals were treated with *C. esculenta* extract, the decrease in body weight was minimised and an improvement in body weight was observed (Table 6).

The present study also indicates that *C. esculenta* can partially inhibit STZ renal toxicity as seen from the blood urea values (Table 7).

A clinically used sulphonylurea, glibenclamide, is known to lower blood glucose levels by stimulating β-cells [15] to release insulin. It is used in the present study to compare the efficacy of the test drug in normal and STZ diabetic rats.

In conclusion, the aqueous extract obtained from *C. esculenta* root exhibits hypoglycemic activity in normoglycemic, oral glucose tolerance and streptozotocin diabetic rats. The further study is under way to elucidate the molecular mechanism of the test drug.

4. Experimental

4.1. Plant material

Root of *Casearia esculenta* was collected from Western ghats of Tamil Nadu and the plant was botanically authenticated and a voucher specimen was deposited in the (AU2145) Department of Botany, Annamalai University, Annamalainagar, Tamilnadu. The plant root was air dried at 25 °C and the dried root was made into fine powder with an auto-mix blender and the powdered part was kept in a deep freezer until the time of use.

4.2. 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 process was repeated 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% solid extract.

4.3. Drugs and chemicals

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

4.4. Animals

Male Wistar albino rats (weighting 140–160 g) were procured form 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*.

4.5. Effect of test drug on blood sugar level in normal fasted rats

Preliminary studies were carried out to determine the time taken to produce peak hypoglycemic effects after oral administration of the test drugs, for each test drug preparation (100, 200, 300 and 400 mg/kg body weight), six animals were used. The drugs were given to animals fasted for 12 h. In all cases, fasting blood sugar level was determined before oral feeding of the drug and, after drug intake, blood sugar levels were determined at half hour intervals for a period of 3 h. These studies showed that peak hypoglycemia occurred 2 h after the administration of the test preparation.

4.6. Effect of the test drug on glucose tolerance in normal rats

Fasted rats were divided into four groups of six animals each, group I serving as control, received only vehicle (3% gum acacia, distilled water) and group II and III received the test drug. Doser of 200 and 300 mg/kg body weight of the extract suspended in vehicle solution were administered orally in a volume of 10 ml/kg body weight. Group IV received glibenclamide (600 μg/kg body weight). All the animals were given glucose (2 g/kg body wt.) 30 min after dosing, blood samples were collected from the tail vein just prior to and 30, 60 and 90 min after the glucose loading. Blood glucose levels were measured.

4.7. Effect of the test drug on streptozotocin- induced diabetic in rats

Rats were made diabetic by a single intraperitoneal injection of streptozotocin (STZ) (50 mg/kg body weight, citrate buffer, 0.1 M, pH 4.5). Then 96 h later blood samples were collected and glucose levels were determined to confirm the development of diabetes. Then, the diabetic rats were divided into five groups of six animals each. Group I received vehicle solution (3% gum acacia, distilled water) orally in a volume of 10 ml/kg body weight. The test drug extract suspended in vehicle was given in doses of 200 and 300 mg/kg body weight orally in a volume of 10 ml/kg body weight to group III and IV. Group V received glibenclamide (600 μg/kg body weight) suspended in vehicle (10 ml/kg weight) Blood samples were collected for the estimation of blood glucose at different time intervals.

In subsequent studies, the test drugs were given orally to separate groups of fasted animals every morning for a period of 15 days. Control groups were run concurrently and these received the vehicle (3% gum acacia) used for preparing the drug doses. Blood sugar level were determined on different day intervals (day 4, day 7 and day 15 after the initiation of experiment.

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