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

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The present study was carried out to evaluate the antihyperglycaemic effect of *Casearia esculenta* root extract and to study the activities of liver hexokinase and gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver and kidney of normal and streptozotocin-induced diabetic rats. Oral administration of aqueous extract of root (300 mg/kg body weight) for 45 days resulted in a significant reduction in blood glucose from 250.79 ± 12.65 to 135.70 ± 8.90 and in a decrease in the activities of glucose-6-phosphatase and fructose-1,6-bishosphatase and an increase in the activity of liver hexokinase. However, in the case of 200 mg/kg body weight of extract, less activity was observed. The study clearly shows that the root extract of *C. esculenta* possesses potent antihyperglycaemic activity but weaker than that of glibenclamide.

1. Introduction

The high prevalance and long-term complication of diabetes mellitus have started an intense search for new oral hypoglycaemic agents from anti-diabetic plants used in traditional medicine. The World Health Organisation has also recommended that this should be encouraged, especially in countries where access to the conventional treatment of diabetes is not adequate [1].

Casearia esculenta Roxb. (Flacourtiaceae) locally known as "Kadala-zhinjil" or "Kottargovai" in Tamil, and "Saptarangi" in Sanskrit is found in many parts of our subcontinent. It is a small tree reaching 6–9 m height, with yellowish white smooth bark, which grows widely in Konkan, East Coast Belt and West Coast, from S. Canara to Cochin. It has been reported to be used by diabetics and decoctions of roots of this plant have been used in rural parts of Tamilnadu as folk medicine for the treatment of diabetes mellitus and piles [2, 3]. A literature survey revealed that Gupta et al. [4] investigated the effects of *C. esculenta* aqueous and alcoholic extracts on fasting blood sugar as well as glucose tolerance and glucose uptake by tissues of rats and rabbits. They reported that the daily feeding of *C. esculenta* did not change the basal fasting blood sugar level significantly. However, the blood sugar was found to be reduced 2 h after the administration of aqueous and alcoholic extracts of the drug in acute experiment and the hypoglycaemic effect of the aqueous extract in normal rats and rabbits was highly significant. Furthermore, Choudhury and Basu [5] have reported the potent hypoglycemic activity of *C. esculenta* root extract prepared from different solvent systems. No systematic experiment was carried out on diabetic animals using this plant extract.

Thus, the present investigation envisages to study the antihyperglycaemic activity of *C. esculenta* root extract and other related biochemical parameters in normal and streptozotocin induced diabetic rats. The effect produced by the drug were compared with those of glibenclamide.

2. Investigations, results and discussion

Changes in blood glucose and glycosylated haemoglobin (HbA_{1c}) in diabetic rats treated with *C. esculenta* and glibenclamide are presented in Table 1. Blood glucose and HbA_{1c} were increased in streptozotocin diabetic rats as compared to normal rats. Administration of *C. esculenta* root extracts (200 mg and 300 mg/kg body wt) and glibenclamide decreased blood glucose and HbA_{1c} as compared to diabetic rats.

Effects on the administration of *C. esculenta,* glibenclamide on hepatic hexokinase and glucose-6-phosphatase, fructose-1,6-bisphosphatase of liver and kidney are presented in Tables 2 and 3. The activity of hepatic hexokinase was significantly decreased while glucose-6-phosphatase and fructose-1,6-bisphosphatase were significantly elevated in streptozotocin treated diabetic rats as compared to normal rats. Administration of *C. esculanta* (200 mg and 300 mg/kg body wt) and glibenclamide increased the activity of hexokinase and decreased the activity of glucose 6-phosphatase and frutose-1,6-bisphosphatase as compared to diabetic rats.

In our study, administration of *C. esculenta* root extracts resulted in a significant reduction in blood glucose levels

Table 1: Effect of *Casearia esculenta* root extract on blood glucose, glycosylated haemoglobin in normal and STZ diabetic rats

Experimental conditions	Blood glucose (mg/dl)		Glycosylated Hb mg/g Hb (45 days of treatment)
	Initial	Final (45 days of treatment)	
Control	66.66 ± 4.25	73.33 ± 4.26[a]	0.24 ± 0.02[a]
Diabetic control	250.76 ± 12.65	311.10 ± 11.91[b]	0.89 ± 0.09[b]
Diabetic + *C. esculenta* root extract (200 mg/kg body wt.)	248.31 ± 8.74	166.66 ± 12.77[c]	0.69 ± 0.08[c]
Diabetic + *C. esculenta* root extract (300 mg/kg body wt.)	250.79 ± 7.77	135.70 ± 8.90[d]	0.46 ± 0.03[d]
Diabetic + glibenclamide (600 ± µg/kg body wt.)	258.72 ± 6.50	119.43 ± 10.03[e]	0.37 ± 0.02[d]

Values are mean ± SD of six rats
Values not sharing a common superscript differ significantly at $P < 0.05$

Table 2: Changes in the activities of hexokinase, glucose-6-phosphatase and fructose-1,6-bisphosphatase in rat liver of different experimental groups

Experimental conditions	Hexokinase (U^a/mg protein)	Glucose-6-phosphatase (U^b/mg protein)	Fructose-1,6-bisphosphatase (U^c/mg protein)
Control	0.25 ± 0.05^a	0.17 ± 0.01^a	0.59 ± 0.12^a
Diabetic control	0.09 ± 0.02^b	0.36 ± 0.03^{bc}	1.22 ± 0.37^b
Diabetic + *C. esculenta* root extract (200 mg/kg body wt.)	0.16 ± 0.05^c	0.32 ± 0.10^c	0.90 ± 0.32^c
Diabetic + *C. esculenta* root extract (300 mg/kg body wt.)	0.22 ± 0.06^{acd}	0.22 ± 0.01^a	0.61 ± 0.19^{acd}
Diabetic + glibenclamide (600 μg/kg body wt.)	0.25 ± 0.05^{ad}	0.19 ± 0.02^a	0.51 ± 0.10^{ad}

Values are mean ± SD of six rats each. Enzyme units are expressed as units/mg protein
[a] μmol of glucose phosphorylated/h
[b] μmol of Pi liberated/min
[c] μmol of Pi liberated/min
Values not sharing a common superscript differ significantly at $P < 0.05$

when compared with diabetic control animals (Table 1). The extract containing 300 mg/kg body weight showed a better glucose level reduction than that with 200 mg/kg body weight. The standard drug glibenclamide showed a better reduction probably due to the crude form of the extracts. As in normal animals [5], *C. esculenta* extracts are certainly capable of reducing blood sugar level even in diabetic animals. The mechanism may be stimulation of β-cell for elevated secretion of insulin, thereby increasing the utilisation of glucose in various tissues.

Glycosylated haemoglobin comprises about 3.4 to 5.8% total haemoglobin in normal human red cells but it is increased in diabetic patients [6]. The amount of this increase is directly proportional to the long lasting fasting blood sugar level. In our study, the glycosylated haemoglobin level was high showing that the diabetic animals had high blood glucose levels. The values decreased very much in *C. esculenta* treated animals showing the influence of the extract on sugar reduction.

Insulin influences the intracellular utilization of glucose in a number of ways. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase, phosphofructokinase and pyruvate kinase. Hexokinase is universally present in cells of all types. Hepatocytes also contain a form of hexokinase called hexokinase D or glucokinase which is more specific for glucose and differs from other forms of hexokinase in kinetic and regulatory properties [7]. Glucokinase (also hexokinase IV) catalyzes the conversion of glucose to glucose-6-phosphate and plays a central role in the maintenance of glucose homeostasis. In the liver, the enzyme is an important regulator of glucose storage and disposal [8]. In our study, the hexokinase activity was decreased in streptozotocin diabetic rats which may be due to insulin deficiency (insulin stimulates and activates hexokinase D). Treatment with *C. esculenta* and glibenclamide elevated the activity of hexokinase D in the liver. *C. esculenta* like glibenclamide may stimulate insulin secretion which may activate hexokinase D, thereby increasing utilization of glucose leading to decreased blood sugar levels. As there is no accumulation of glucose-6-phosphate, hexokinase activity is not inhibited by auto-regulation, as in the case of diabetic rats. Hence the activity of hexokinase D is decreased in *C. esculenta* and glibenclamide treated diabetic rats.

Insulin decreases gluconeogenesis by decreasing the activities of key enzymes, such as glucose-6-phosphatase, fructose-1,6-bisphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase [9]. In our study, the increased activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver and kidney of the streptozotocin diabetic rats may be due to insulin insufficency. In *C. esculenta* and glibenclamide treated groups, the activities of these two enzymes were significantly reduced, this may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic key enzymes.

Thus, in this study *C. esculenta* herbal medicine is shown to exhibit antihyperglycemic activity. However, the effect of the crude extract is weaker than that caused by the standard drug glibenclamide. Further work is underway to separate the active principle and show its efficacy.

Table 3: Changes in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in kidney of different experimental groups

Experimental conditions	Glucose-6-phosphatase (U^a/mg protein)	Fructose-1,6-bisphosphatase (U^b/mg protein)
Control	0.21 ± 0.01^a	0.79 ± 0.02^a
Diabetic control	0.30 ± 0.02^b	1.14 ± 0.17^b
Diabetic + *C. esculenta* root extract (200 mg/kg body wt.)	0.24 ± 0.01^c	1.02 ± 0.01^c
Diabetic + *C. esculenta* root extract (300 mg/kg body wt.)	0.21 ± 0.01^a	0.80 ± 0.03^a
Diabetic + glibenclamide (600 μg/kg body wt.)	0.21 ± 0.01^a	0.81 ± 0.02^a

Values are mean ± SD of six rats each. Enzyme units are expressed as units/ mg protein
[a] μmol of glucose phosphorylated/h
[b] μmol of Pi liberated/min
[c] μmol of Pi liberated/min
Values not sharing a common superscript differ significantly at $P < 0.05$

3. Experimental

3.1. Plant material

Roots of *Casearia esculenta* were collected from Western ghats of Tamil Nadu and the plant was botanically authenticated. 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 the aqueous extract

Dry fine powder (100 g) was suspended in 250 ml water for 2 h and then heated at 60–65 °C for 30 min (since a boiled decoction of root of this plant has been used as remedy for diabetes). The extract was preserved and the process was 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. Upon evaporation at 40 °C, the filtrate yielded 24% solid extract.

3.3. Drugs and chemicals

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

3.4. Animals

Male Wistar albino rats (weighing 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 fed on standard diet (Hindustan Liver, Bangalore) and water *ad libitum*.

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 hyperglycaemia 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 were injected with 2% gum acacia alone that served as control.

3.6. Study design

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 receiving *Casearia esculenta* root extract (200 mg/kg body weight) in 2% gum acacia using an intragastric tube daily for 45 days.
Croup 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 receiving glibenclamide orally (600 μg/kg body weight) as aqueous solution using an intragastric tube daily for 45 days.

At the end of the experiment, the animals were killed by cervical dislocation. Blood was collected in oxalate–fluoride mixture for blood glucose estimation [10] and liver and kidney was excised immediately and homogenised in ice-cold, 0.1 M Tris-HCl buffer. The homogenate was used for the estimation of tissue protein [11] and RBC was used for the estimation of glycosylated haemoglobin [12].

3.7. Enzyme assays

3.7.1. Hexokinase (EC 2.7.1.1)

Hexokinase was assayed according to Brandstrup et al. [13] by estimation of residual glucose in the reaction supernatant. The reaction mixture contained glucose solution (0.005 M), ATP (0.72 M), magnesium chloride (0.05 M), dipotassium hydrogen phosphate (0.0125 M), potassium chloride (0.1 M), sodium fluoride (0.5 M) and Tris-HCl buffer (0.01 M, pH.8.0). The reaction was initiated by the addition of an aliquot of tissue homogenate. An aliquot of the reaction mixture was taken immediately (zero time) to tubes containing TCA (trichloroacetic acid). A second aliquot was removed after 30 min of incubation at 37 °C and added to tubes containing TCA. The precipitated protein was removed by centrifugation and the residual glucose in the supernatant was estimated by the o-toluidine method of Sasaki et al. [10]. The difference between the two values gave the amount of glucose phosphorylated. The enzyme activity was expressed as μmol of glucose-6-phosphate formed/h/mg protein.

3.7.2. Glucose-6-phosphatase (EC 3.1.3.9)

Glucose-6-phosphatase was assayed according to Koide and Odo [14] by estimation of inorganic phosphate liberated from glucose-6-phosphate (G6P). The incubation mixture contained malic acid buffer (0.1 M, pH 6.5), glucose-6-phosphate (0.01 M) and tissue homogenate. The reaction mixture was incubated at 37 °C for 1 h. TCA was added to terminate the enzyme activity then centrifuged and the phosphate content of the supernatant was estimated by the Fiske and Subbarow method [15], in which an aliquot of supernatant, ammonium molybdate and ANSA (1-amino-2-napthol-4-sulphonic acid) dissolved in sodium bisulphate and sodium sulphite were taken. The blue colour developed was read after 20 min at 620 nm. Enzyme activity was expressed as μmole of Pi liberated/min/mg protein.

3.7.3. Fructose-1,6-bisphosphatase (EC 3.1.3.11)

Fructose-1,6-bisphosphatase was assayed by the method of Gancedo and Gancedo [16]. The assay medium contained Tris-HCl buffer (0.1 M, pH 7.0) fructose-1,6-bisphosphate (0.05 M), magnesium chloride (0.1 M), potassium chloride (0.1 M), EDTA (0.001 M) and tissue homogenate as enzyme source. The incubation was carried out at 37 °C for 15 min and the reaction was terminated by the addition of TCA. The suspension was centrifuged and phosphorus content of the supernatant was estimated as described above [15]. The enzyme activity was expressed as μmol of glucose-6-phosphate formed/min/mg protein.

3.8. Statistical analysis

Values were represented as mean ± SD, Data were analysed using Analysis of Variance (ANOVA) and group mean were compared with Duncan's multiple range test (DMRT).

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