

EFFECT OF *CASEARIA ESCULENTA* ROOT EXTRACT ON BLOOD GLUCOSE AND PLASMA ANTIOXIDANT STATUS IN STREPTOZOTOCIN DIABETIC RATS

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Effect of Casearia esculenta root extract on blood glucose and plasma antioxidant status in streptozotocin diabetic rats. A. PRAKASAM, S. SE-
THUPATHY, K.V. PUGALENDI. Pol. J. Pharmacol., 2003, 55, 43–49.

Our preliminary study shows that an oral administration of an aqueous extract of *Casearia esculenta*, an indigenous antidiabetic plant popularly used in South India for diabetes mellitus, lowers blood glucose level under normal and glucose load conditions, and in streptozotocin (STZ)-induced diabetes in rats. The study was further undertaken to evaluate the antioxidant potential of *C. esculenta* in STZ diabetic rats. Oral administration of *C. esculenta* root extract at doses of 200 and 300 mg/kg for 45 days resulted in significant reduction in plasma thiobarbituric acid reactive substances (TBARS), hydroperoxide and ceruloplasmin and a significant elevation in plasma reduced glutathione (GSH), ascorbic acid (vitamin C) and α -tocopherol (vitamin E). The study indicates that *C. esculenta* root extract at doses of 200 and 300 mg/kg restored all the antioxidant parameters to near normal value.

Key words: antioxidants, *Casearia esculenta*, diabetes mellitus, streptozotocin, TBARS

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INTRODUCTION

In the Indian system of medicine (Ayurvedic and Siddha), many plants are claimed to possess antidiabetic activity. In practice, it is being increasingly recognized to be an alternative approach to modern medicine. The World Health Organization (WHO) has also recommended that this practice should be encouraged, especially in countries where access to conventional treatment of diabetes mellitus is not adequate [32].

Currently, oxidative stress is suggested as a mechanism underlying diabetes and diabetic complications [14], which results from an imbalance between radical generating and radical scavenging systems. In diabetes, protein glycation and glucose autoxidation may generate free radicals, which in turn catalyze lipid peroxidation [4, 23]. Many studies carried out in recent years have shown elevated lipid peroxidation in diabetic animals [9], impaired GSH metabolism, [21] and decreased ascorbic acid level [17].

The level of lipid peroxidation in cell is controlled by various cellular defense mechanisms consisting of enzymatic and nonenzymatic scavenging systems [15]. The efficiency of this defense mechanism is altered in diabetes [33] and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue injury.

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 plateau and South India. In Indian traditional medicine, the plant has been popular remedy for the treatment of diabetes mellitus [1, 31, 34] and our study drug is one of the major ingredients of D-400, a largest selling antidiabetic drug in India (Himalaya Drug Co., Bangalore) [22]. The first scientific study undertaken by Gupta et al., [12] demonstrated the hypoglycemic effect of this plant in rat and rabbits, and Choudhury and Basu [7] then reported that *C. esculenta* root extract did contain hypoglycemic factor(s) which reduced blood sugar level in experimental animals. Our preliminary experimental results were highly encouraging as they revealed that blood glucose level was significantly lowered after oral administration of *C. esculenta* root extract under normal, and glucose load conditions and in streptozotocin (STZ)-induced diabetes. To our knowledge, no detailed investigations had

been carried out to shed light on the antioxidant property of *C. esculenta* root extract. Thus, the present investigation envisages confirming the antidiabetic effect of *C. esculenta* root extract and examining the effect of the test drug on plasma antioxidant status.

MATERIALS and METHODS

Male Wistar albino rats (weighting 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 were fed on standard diet (Hindustan Liver, Bangalore) and water *ad libitum*. All studies were conducted in accordance with the National Institutes of Health guide [24].

STZ was obtained from Sigma Chemical Co. (USA) and all other chemical used were of analytical grade. Root of *Casearia esculenta* was collected from Western ghats of Taminladu and the plant was botanically authenticated by Dr. C. Chelladurai, Research Officer, Survey Medicinal Plant Unit (S.M.P), Central Council for Research in Siddha and Ayurvedic, Siddha Medical College, Palayamkottai, Tamilnadu. 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 ground into fine powder with auto-mix blender and the powdered part was kept in deep freezer until the time of use.

The 100 g of dry fine powder was suspended in 250 ml of 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% semi-solid extract.

Preliminary studies were carried out to determine the time necessary to produce peak hypoglycemic effect after oral administration of the test drugs. For each test drug preparation (100, 200, 300 and 400 mg/kg), 6 animals were used. The drugs were given to animals fasted for 12 h. In all cases, fasting blood sugar level was determined before oral administration of the drug, and after drug in-

take, blood sugar levels were estimated at half-an-hour intervals for a period of 3 h. These studies showed that peak hypoglycemia occurred 3 h after the administration of the test preparation.

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

Rats were made diabetic by a single intraperitoneal injection of STZ (50 mg/kg, dissolved in 0.1 M citrate buffer, pH 4.5). Ninety six hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Albino rats with blood glucose level between 240–260 mg/dl were used in the experiment. The diabetic rats were divided into five groups of six animals each. Six rats were injected with 2% gum acacia alone that served as group I – control. Group II received vehicle solution (2% gum acacia) orally in a volume of 10 ml/kg. The test drug extract suspended in vehicle was given at doses of 200 and 300 mg/kg orally in a volume of 10 ml/kg to group III and IV, respectively, and group V received glibenclamide (600 µg/kg) suspended in vehicle (10 ml/kg). Blood samples were collected at different time intervals (at 30 min, 60 min and 90 min) for the estimation of blood glucose and the experiment was continued further to study the ef-

fect of *C. esculenta* root extract on plasma antioxidant status in STZ diabetic rats.

At the end of the experiment (45 days of treatment), blood was collected into heparinized tubes, the plasma was separated by centrifugation, and the following biochemical parameters were determined in our laboratory. Blood glucose level was measured by o-toluidine method [28], plasma TBARS content was estimated by the method of Nichans and Samuelson [25], and OGTT [8], hydroperoxide [18], ceruloplasmin [27], reduced glutathione [5], α-tocopherol [3] and ascorbic acid [26] were determined on the same day according to the procedures described previously.

Percent of glycemic changes was calculated as a time function, by applying the formula [19]:

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

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

Two-way analysis of variance (ANOVA) with interaction effects was employed for analyzing the initial dose-response data (Tab. 1–3), and one-way analysis of variance was employed for analyzing the antioxidant status and general parameters (Tab. 4–6). Inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS package was used for analysis.

RESULTS

Administration of *C. esculenta* root extract was found to reduce blood glucose level in normal rats (Tab. 1). The maximum reduction in blood glucose

Table 1. Effect of *C. esculenta* root extract on blood glucose level in fasted normal rats. Values are means ± SD of six animals in each group

Group	Treatment mg/kg <i>po</i>	Blood glucose(mg/dl)			
		Fasting	1 h	2 h	3 h
I	Control – received vehicle only	64.3 ± 3.7	64.3 ± 2.1	62.7 ± 3.3	64.3 ± 2.1 ^a
II	<i>C. esculenta</i> 100	67.3 ± 3.8	65.1 ± 2.4	63.5 ± 2.4	62.7 ± 1.2 ^a
III	<i>C. esculenta</i> 200	64.3 ± 5.7	59.5 ± 2.1 ^a	54.0 ± 2.4 ^a	47.7 ± 4.2 ^b
IV	<i>C. esculenta</i> 300	69.0 ± 2.1	65.1 ± 2.4	60.3 ± 6.5	45.6 ± 6.5 ^b
V	<i>C. esculenta</i> 400	65.4 ± 4.2	66.7 ± 0.1	63.5 ± 2.4	55.3 ± 2.4 ^c

Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

was noted 2 h after the administration of the extract. The reduction was maximum at the dose of 300 mg/kg. *C. esculenta* root extract at doses of 200 and 300 mg/kg was taken for further studies.

The effect of *C. esculenta* extract on glucose tolerance is presented in Table 2. In glucose-fed rats (2 g/kg), administration of 200 and 300 mg/kg of *C. esculenta* extract significantly increased the tolerance for glucose. The maximum glucose tolerance

was noted for the test dose of 300 mg/kg after glucose loading (3 h after drug dosing).

The effect of *C. esculenta* root extract in STZ-induced diabetes in rats is given in Table 3. The fasting blood glucose level in STZ diabetic rats was 240–260 mg/dl. The initial reduction in blood glucose was observed 2 h after the administration of *C. esculenta* extract. A reduction in body weight was observed in STZ diabetic animals, but when the

Table 2. Effect of *C. esculenta* root extract on oral glucose (2 g/kg of body weight) tolerance in rats. Values are means \pm SD of six animals in each group

Group	Treatment g/kg po	Blood glucose (mg/dl)			
		Fasting	30 min	60 min	90 min
I	Control + glucose – 2.0	69.0 \pm 2.1	146 \pm 8.9	130 \pm 4.9	122.2 \pm 9.4
II	<i>C. esculenta</i> 0.2 + glucose – 2.0	69.8 \pm 2.5	125.4 \pm 5.0 ^a	114.3 \pm 4.2 ^a	93.6 \pm 5.0
III	<i>C. esculenta</i> 0.3 + glucose – 2.0	64.3 \pm 7.7	127.0 \pm 2.4 ^a	114.3 \pm 4.3 ^a	83.3 \pm 2.1 ^a
IV	Glibenclamide 0.0006 + glucose – 2.0	65.1 \pm 2.5	128.6 \pm 4.3 ^a	103.2 \pm 2.5 ^a	76.2 \pm 4.3 ^a

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 sugar in normal and STZ diabetic rats. Values are means \pm SD of six animals in each group

Group	Treatment mg/kg po	Blood glucose (mg/dl)			
		Fasting	1 h	2 h	3 h
I	Control (2% gum acacia)	66.6 \pm 4.25	65.7 \pm 3.0 ^a (–1.38)	64.8 \pm 2.6 ^a (–2.85)	63.8 \pm 3.0 ^a (–4.32)
II	Diabetic control	250.2 \pm 8.2 ^a	247.1 \pm 5.3 ^{bc} (–1.25)	241.2 \pm 5.3 ^{bc} (–3.60)	239.2 \pm 3.0 ^b (–4.39)
III	Diabetic + <i>C. esculenta</i> 200	248.3 \pm 8.8 ^a	241.2 \pm 5.2 ^b (–2.87)	237.2 \pm 3.0 ^b (–4.45)	233.3 \pm 3.0 ^c (–6.02)
IV	Diabetic + <i>C. esculenta</i> 300	253.9 \pm 7.8 ^a	250.9 \pm 13.2 ^{bc} (–1.17)	241.1 \pm 10.5 ^{bc} (–5.0)	224.4 \pm 5.3 ^{cd} (–11.63)
V	Diabetic + glibenclamide 0.6	258.7 \pm 6.5 ^a	252.9 \pm 10.5 ^c (–2.23)	239.2 \pm 13.2 ^c (–7.54)	225.5 \pm 8.0 ^d (–12.84)

Values in parenthesis indicate the percentage glycemic changes. Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

Table 4. Body weight, body weight changes, food intake and water intake in control and experimental animals. Values are given as means \pm SD of six animals in each group

Group	Treatment mg/kg po	Body weight (g)		Body weight changes (g)	Food intake g/week	Water intake L/week
		Initial	Final			
I	Control (2% gum acacia)	162.5 \pm 2.7	202.5 \pm 6.9 ^a	+ 40.0 \pm 7.7	82.1 \pm 3.20 ^a	4.34 \pm 0.35
II	Diabetic control	165.8 \pm 3.8	150.8 \pm 3.8 ^b	–15.0 \pm 5.5	76.8 \pm 1.75 ^a	7.12 \pm 0.44
III	Diabetic + <i>C. esculenta</i> 200	160.8 \pm 2.0	163.3 \pm 2.6 ^c	+3.3 \pm 3.4	79.4 \pm 2.70 ^{ab}	6.66 \pm 0.31 ^a
IV	Diabetic + <i>C. esculenta</i> 300	159.2 \pm 2.1	163.3 \pm 2.6 ^c	+5.8 \pm 2.6	87.6 \pm 2.70 ^c	6.42 \pm 0.24 ^a
V	Diabetic + glibenclamide 0.6	161.7 \pm 2.6	167.5 \pm 5.2 ^c	+6.6 \pm 4.1	87.2 \pm 3.56 ^c	5.80 \pm 0.31

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 extract on blood glucose, plasma TBARS, hydroperoxide and ceruloplasmin in control and experimental animals. Values are given as means \pm SD of six animals in each group

Group	Treatment mg/kg po	Blood glucose (mg/dl)		TBARS (nmol/ml)	Hydroperoxide (nmol/ml)	Ceruloplasmin (mg/dl)
		Initial	Final			
I	Control (2% gum acacia)	66.66 \pm 4.25	73.33 \pm 4.26 ^a	2.1 \pm 0.5 ^a	0.9 \pm 0.2 ^a	23.0 \pm 4.3 ^a
II	Diabetic control	250.76 \pm 12.65	311.10 \pm 11.91 ^b	3.0 \pm 0.6 ^b	1.6 \pm 0.1 ^b	38.8 \pm 4.6 ^b
III	Diabetic + <i>C. esculenta</i> 200	248.31 \pm 8.74	166.66 \pm 12.77 ^c	2.7 \pm 0.7 ^{bc}	1.6 \pm 0.2 ^b	30.8 \pm 4.9 ^c
IV	Diabetic + <i>C. esculenta</i> 300	250.79 \pm 12.65	135.70 \pm 8.90 ^d	2.3 \pm 0.5 ^{ac}	1.0 \pm 0.2 ^a	27.8 \pm 6.2 ^c
V	Diabetic + glibenclamide 0.6	258.72 \pm 6.50	119.43 \pm 10.03 ^e	2.1 \pm 0.4 ^a	0.9 \pm 0.2 ^a	26.6 \pm 4.4 ^c

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 plasma GSH, α -tocopherol and ascorbic acid in control and experimental animals. Values are given as means \pm SD of six animals in each group

Group	Treatment mg/kg po	GSH (mg/dl)	-Tocopherol (mg/dl)	Ascorbic acid (mg/dl)
I	Control (2% gum acacia)	23.6 \pm 1.8 ^a	1.0 \pm 0.2 ^a	1.9 \pm 0.2 ^a
II	Diabetic control	15.4 \pm 2.1 ^b	0.6 \pm 0.1 ^b	1.1 \pm 0.2 ^b
III	Diabetic + <i>C. esculenta</i> 200	18.7 \pm 2.1 ^c	0.7 \pm 0.1 ^b	1.4 \pm 0.3 ^b
IV	Diabetic + <i>C. esculenta</i> 300	21.6 \pm 2.3 ^a	1.0 \pm 0.1 ^a	1.7 \pm 0.3 ^a
V	Diabetic + glibenclamide 0.6	23.2 \pm 2.4 ^a	1.0 \pm 0.1 ^a	1.8 \pm 0.2 ^a

Values not sharing a common superscript differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT)

animals were treated with *C. esculenta* extract, the decrease in body weight was minimized to almost nil and the improvement in body weight was observed (Tab. 4).

Table 5 shows the levels of blood glucose, thiobarbituric acid reactive substances (TBARS), hydroperoxide and ceruloplasmin in normal and diabetic rats. The plasma TBARS, hydroperoxide and ceruloplasmin levels are significantly elevated in diabetic rats as compared to normal rats. Administration of *C. esculenta* extract (200 and 300 mg/kg) or glibenclamide induced significant reduction in plasma hydroperoxide and ceruloplasmin content as compared to diabetic rats.

Table 6 shows the plasma GSH, α -tocopherol and ascorbic acid levels which are significantly lower in diabetic rats than the normal controls, Administration of *C. esculenta* (200 and 300 mg/kg) or glibenclamide increased significantly the GSH, α -tocopherol and ascorbic acid levels as compared with diabetic rats.

DISCUSSION

The aim of the present study was to confirm the antidiabetic effect and to evaluate the antioxidant potential of *C. esculenta* medicinal plant in STZ diabetic rats. Our results show that aqueous extract of *C. esculenta* reduces the blood glucose level in normal, glucose-loaded and STZ diabetic rats.

Oral administration of *C. esculenta* root extract significantly reduces blood glucose level in normal rats and the test drug also remarkably improves oral glucose tolerance in normal rats. At the present juncture, it is not possible to pinpoint the mechanism of anti-hyperglycemic action of the extract of *C. esculenta*. However, based on an earlier report, some suggestion can be made for its possible mechanism. It has been reported that an infusion of *C. esculenta* extract inhibits blood glucose absorption from the gut [20]. Thus, a possibility that retardation of intestinal glucose absorption may also be

partially responsible for inhibition of hyperglycemia in glucose-fed rats.

It has further been noted from earlier *in vitro* studies with rat hemi-diaphragm that prolonged treatment with *C. esculenta* drug rendered the tissue more sensitive to insulin. The increased sensitivity 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 [21]. Since the blood glucose-lowering effect of the extract of *C. esculenta* was observed in fasted normal as well as STZ diabetic rats, this effect could, possibly be due to the increased peripheral glucose utilization.

Oral administration of an aqueous extract of *C. esculenta* root (at doses of 200 and 300 mg/kg) has shown antioxidant effect in STZ-induced diabetes in rats. In the present study, we have observed an increase in the levels of plasma TBARS which is an index of lipid peroxidation, and hydroperoxide in the STZ diabetic rats. These results confirm the possibility that the major function of the extract is the protection of vital tissues including liver, kidney, brain and pancreas, thereby reducing the causation of diabetes. As others have reported [11, 14], an increase in the levels of lipid peroxides and TBARS in plasma is generally thought to be the consequence of increased production and liberation into the circulation.

The antioxidant defense system is significantly altered in diabetes. Ceruloplasmin is a copper-containing oxidase, which serves to transport copper in tissues. Ceruloplasmin has been established as chain-breaking antioxidant with a potential to scavenge peroxy radicals [13]. The level of ceruloplasmin was significantly increased in diabetic rats when compared to control rats which may facilitate the scavenging action on peroxy radicals.

Vitamin C is an excellent water-soluble antioxidant that primarily scavenges oxygen radicals. Vitamin C has been reported to contribute to up to 24% of the total peroxy radical-trapping antioxidant activity (TRAP) [2]. We have observed a decreased level of plasma vitamin C in the diabetic rats. This decreased level could be due to the increased utilization of vitamin C in deactivation of the increased levels of reactive oxygen species or to the decrease in the GSH level, since the GSH is required for the recycling of vitamin C [6, 16].

Glutathione (GSH) is a metabolic regulator and putative indicator of health. We observed lower

level of plasma GSH in STZ diabetic rats. It appears that generation of oxygen radicals by increased levels of glucose causes increased utilization of GSH. Other workers have also reported decreased level of plasma GSH in STZ diabetic rats [10, 21]

The most important antioxidant in the cell membrane is α -tocopherol, it interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxy radicals, thus protecting the cell structures against damage [29, 30]. The decreased level of α -tocopherol found in the diabetics as compared with control rats could be due to the increased oxidative stress which accompanies the decrease in the level of antioxidants, and may be related to the causation of diabetes mellitus. In this context, Garg et al. [10] reported the decreased level of plasma α -tocopherol in STZ diabetic rats.

A reduction in body weight was observed in rats with STZ-induced diabetes, but when the animals were treated with *C. esculenta* extract, the decrease in body weight was minimized to almost nil and the improvement in body weight was observed

In conclusion, we strongly report the hypoglycemic effect of *C. esculenta* in normal and STZ-treated animals. It has been found that only 300 mg/kg of *C. esculenta* extract exhibited significant antioxidant effect. Thus, the root extract of *C. esculenta* offers an antioxidant protection in STZ-induced diabetes in rats.

Acknowledgment. The authors are grateful to Rameshwardasji Birla Smarak Kosh for providing fellowship assistance.

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Received: August 21, 2002; in revised form: November 21, 2002.