Iranian Journal of Pharmaceutical Research (2006) 1: 47-51
Received: December 2004
Accepted: October 2005

Original Article

Hypoglycemic Activity of *Casearia esculenta* Roxb. in Normal and Diabetic Albino Rats

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Abstract

The hypoglycemic effect of ethanolic extract of *Casearia esculenta* was investigated on alloxan induced diabetic rats. The blood glucose levels were measured at 0, 1, 2 and 3 h after the treatment. The ethanolic extract of *C. esculenta* (250 mg/kg) reduced the blood glucose of normal rat from 85.50±1.22 to 64.67±3.27 mg/dl, 3 h after oral administration of the extract ($P<0.05$). It also significantly lowered blood glucose level in alloxan induced diabetic rat from 331.67±4.90 to 130.33±6.53 mg/dl, 3 h after oral administration of the extract ($P<0.05$). The antihyperglycemic activity of *C. esculenta* was compared with tolbutamide, an oral hypoglycemic agent.

Keywords: *C. esculenta*; Antihyperglycemic; Hypoglycemic; alloxan.

Introduction

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and 1.3 % of the population suffers from this disease throughout the world (1). These metabolic disorders include alteration in the carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretions and/or insulin action. The characteristic symptoms of diabetes are polyuria, polydypsia, polyphagia, and unexpected weight loss. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes, which are the major causes of morbidity and death (2). Many herbal products, including several metals and minerals have been described for the cure for diabetes mellitus in ancient literature. There is an increasing demand by patients to use the natural products with antidiabetic activity due to side effects associated with the use of insulin and oral hypoglycemic agent such as sulfonylureas, metformin, α-glucosidase inhibitors, troglitazone, etc (3-6). Herbal preparations alone or in combination oral hypoglycemic agents some time produce a good therapeutic response in some resistant cases where modern medicines alone fail (7). The available literature shows that there are more than 400 plant species showing hypoglycemic activity (8-10) and presently several laboratories are involved in isolating new herbal hypoglycemic agents. Though some of the plants are reputed in the indigenous system of medicine for their activities, it remains to be scientifically established.

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C. esculenta a small tree belonging to the family of Samydaceae, reaching 6-9 m in height, frequently met with in peninsular India in the eastern and Western ghats upto 1,200 m, in the coastal plains, and in the hills of North-eastern India (11). The root is used as antipyretic, tonic to the liver, diseases of the blood, leucoderma, bronchitis, and asthma (12). The traditional medical practitioners of Kolli hills, Tamilnadu, are also using this plant to cure various ailments. The present study focused to evaluate ethanolic extract of *C. esculenta* in normal and alloxan-induced diabetic rats.

Experimental

Plant material

The roots of the plant were collected from the Hogenakkal region, Dharmapuri District in the month of April and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Salem. A voucher specimen (CEA-1) has been kept in our museum for future reference. The roots were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of roots of *C. esculenta* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform: water (13). After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Wistar rats of either sex and of approximately the same age, weighing about 150-175 g, obtained from Perundurai Medical College, Perundurai, were used for the study. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. The animals were exposed to alternate cycle of 12 h of darkness and light each. Before each test, the animals were fasted for atleast 12 h. The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same.

Acute toxicity studies

Swiss albino mice of either sex and of approximately the same age, weighing about 20-30 g were used for the acute toxicity studies (14). The animals were divided into control and test groups containing six animals each. The control group received the vehicle (1 % acacia gum) while the test groups got graded doses of different extracts orally and were observed for mortality till 48 h and the LD_{50} was calculated.

Induction of diabetes

Animals were fasted for 24 hours and injected with freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg, i.p.) as reported previously (15-17). After a week, rats with marked hyperglycemia (fasting blood glucose >300 mg/dl) were employed for the study.

Effect of C. esculenta on blood glucose level in rats

Different groups of rats were used to study the effect of ethanolic extract of *C. esculenta*. The animals were divided into five groups each consisting of 6 rats. First and second groups were served as untreated, normal control and diabetic control and fed on distilled water alone. Third and fourth groups were normal rats and diabetic rats respectively, treated with ethanolic extract (250 mg/kg) of roots of *C. esculenta*. Fifth group was diabetic rats treated with 100 mg/kg of tolbutamide, an oral hypoglycemic

Table 1. Effect of ethanolic extract of *C. esculenta* on fasting blood glucose levels in normal and diabetic rats

Groups	Dose (mg/kg)	Fasting blood glucose at different hours after the treatment (mg/dl)			
		0 h	1 h	2 h	3 h
Untreated normal	-	84.17±1.19	85.33±1.31	85.17±1.55	86.17±1.88
Treated normal	250	85.50±1.22	72.67±1.63	70.50±2.37*	64.67±3.27*
Untreated diabetics	-	332.50±7.11	334.33±6.53	338.67±5.22	340.50±3.51
Treated diabetics	250	331.67±4.90	224.33±5.72*	195.50±7.11*	130.33±6.53*
Tolbutamide	100	328.67±5.22	185.50±4.98*	130.33±5.72*	112.67±4.41*

Values are expressed as mean ± SEM, n=6, * P<0.05 when compared with control.

agent. After an overnight fast, the plant extract suspended in 5 % gum acacia was fed by gastric gavage. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2 and 3 hours (18, 19) after feeding the plant extracts. The blood glucose level was determined by the O-toluidine method of Fings et al (20).

Plasma insulin was assayed by the modified method of Herbert et al (21) using insulin radioimmunoassay kit obtained from BARC, Mumbai, India. Dextran coated charcoal in 0.2 M glycine buffer was used to separate bound and free insulin. Standard curve range was 3.9–250 µU/ml insulin.

Statistical analysis

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul's multiple range test and differences below P<0.05 are considered as significant.

Results and discussion

The roots of *C. esculenta* were collected and extracted. The average percentage yield of alcohol extract of *C. esculenta* was found to be 2.6 % *w/w*. The preliminary phytochemical screenings of the ethanolic extract of roots of *C. esculenta* revealed the presence of alkaloids, glycosides, saponins, phytosterols, tannins and amino acids. The LD_{50} of the ethanolic extract was found to be 2495 mg/kg.

The effect of ethanolic extract of *C. esculenta* on fasting blood glucose level was assessed in normal and diabetic rats at various time intervals are shown in Table 1. The mean blood glucose level was decreased from 85.50±1.22 to 64.67±3.27 mg/dl in normal rats treated with ethanolic extract of *C. esculenta* (P<0.05). A significant decrease in blood glucose level was observed in the diabetic group treated with *C. esculenta* from an initial level of 331.67±4.90 to 130.33±6.53 mg/dl, 3 hours after administration extract, which is comparable to that of effect of 100 mg/kg of tolbutamide.

The effect of ethanolic extract of *C. esculenta* on plasma insulin levels in normal and diabetic rats is shown in Table 2. The plasma insulin levels in diabetic group were decreased significantly compared to that of normal groups. In the diabetic rats with the treatment of ethanolic extract of *C. esculenta*, the insulin levels were significantly increased to 18.33±0.82 µU/ml from an initial value of 8.67±0.49 µU/ml. In the normal treated rats also there was a slight increase in insulin

Table 2. Effect of alcoholic extract of *C. esculenta* on plasma insulin in normal and diabetic rats.

Groups	Dose (mg/kg)	Plasma insulin (µU/ml)	
		Before treatment	After treatment (3 h)
Untreated normal	-	24.33±0.82	23.67±1.96
Untreated diabetics	-	8.83±0.90	7.67±1.14
Treated normal	250	22.0±1.31	28.67±1.63*
Treated diabetics	250	8.67±0.49	18.33±0.82*
Tolbutamide	100	8.33±1.14	25.17±1.06*

Values are expressed as mean ± SEM, n=6, * P<0.05 when compared with control.

levels after the treatment with ethanolic extract of *C. esculenta*.

In this study the ethanolic extract of *C. esculenta* at the dose of 250 mg/kg produced a significant fall in the blood glucose level in both normal and diabetic rats and this was evident 2 hours after the administration of the extracts. On the other hand, tolbutamide caused significantly more hypoglycemia in comparison with the plant extract. Emphasize is laid on glucose homeostasis as a severe hypoglycemia can result in life threatening situation. Therefore, lesser hypoglycemia with plant extract in comparison with tolbutamide is a desirable feature. The antihyperglycemic activity of ethanolic extract of *C. esculenta* was associated with an increase in plasma insulin levels, suggesting that the activity could be due to insulinogenic activity of the extract. The increased levels of insulin in diabetic treated rats in this study, indicate that the ethanolic extract of *C. esculenta* stimulates insulin secretion from the remanent β cells or/and from regenerated β cells. Similar effect, i.e. insulinogenic activity with the treatment of some medicinal plants was shown by Karunanayake et al. (22) and Cakici et al. (23). Further investigation is expected to characterize the active hypoglycemic principle. In our studies the ethanolic extract of *C. esculenta* produced the maximum glucose lowering activity in diabetic rats after 3 hours and produced significant hypoglycemic activity in normal rats.

Acknowledgement

We thank Dr. B. Jayakar, Principal, Vinayaka Mission's College of Pharmacy, Salem, for his encouragement and support throughout the work.

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