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RESEARCH ARTICLE

Hypoglycaemic Activity of Ethanol Extract of *Cinnamomum tamala* Leaves in Normal and Streptozotocin Diabetic Rats

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ABSTRACT

Indian traditional system of medicine has been used plants and herbs for treatment of diabetes since time of Vedic glory. In this regard many plants are still untouched, having remarkable efficacy. The blood glucose lowering effect of 95% ethanol extract of *Cinnamomum tamala* (Hamm.) Nees. & Eberm., leaves was investigated at a single oral dose of 250mg/kg body weight in normal fasted, fed, glucose loaded and streptozotocin-induced diabetic male albino rats. Further, effect of crude extract was studied on muscle glycogen content and histology of pancreatic β-cells granularity of normal rats to probe in to its mechanism of action. Significant reduction in blood glucose levels observed in fasted, fed and diabetic rats. Extract also suppressed the peak value significantly in the glucose loaded rats. Marked degranulation in pancreatic β-cells of extract treated rats associated with corresponding blood glucose lowering suggests insulin secretogogue effect of extract that promote the peripheral utilization of glucose and also increase the muscle glycogen store in fed model, resulting in hypoglycaemic response. In diabetic model the action of *C. tamala* extract may be insulin like.

Keywords: *Cinnamomum tamala (Hamm.) Nees. & Eberm., β-cells, Hypoglycaemic, Insulin release, Streptozotocin-induced diabetes*

Cinnamomum tamala (Hamm.) Nees. & Eberm. (Lauraceae) commonly known as Tejpat (Fig. 1) is a small evergreen tree indigenous in India [1]. Plant is distributed in tropical and sub-tropical Himalaya and cultivated as spice in India [2]. It has been used in Indian folk medicine as tonic to brain, anthelmintic, diuretic and treating diseases of the anus and rectum [3].

Aqueous extract of leaves has been significantly lowered the blood sugar in fasted and glucose loaded fasted rabbits [4]. Oral administration of leaves powder before taking a selective diet was found to produce hypoglycaemic effect along with insulin release from pancreatic β-cells of type 2 diabetic patients [5]. An alcoholic fraction (210mg/kg) of leaves was produced blood sugar lowering response in fasted rats [6]. Long term application of alcoholic extract of leaves was lowered the blood sugar in alloxan-diabetic rats [7]. However, we have not found any studies on blood glucose lowering efficacy of *C. tamala* using the experimental models of different backgrounds of blood glucose levels in combined and also the veracity of previous claims has not been established the mechanism of action to justify the anti-diabetic potential of the Tejpat leaves. Thus, in the present systematic investigation we have evaluated the hypoglycaemic activity of *C. tamala* extract in fasted, fed, glucose loaded, and streptozotocin-induced diabetic rats along with its effect on muscle glycogen content and histology of insulin producing pancreatic β-cells of islets of Langerhans of normal rats to probe in to its mechanism of action.

MATERIALS AND METHODS

Plant Material

C. tamala leaves (Fig. 2) were collected fresh in month of December, from a village *Bakshi Ka Taalab* (latitude 26°59'39''N and longitude 80°54'7''E) of Lucknow, India, followed intensive care [8]. Plant was botanically identified and authenticated by Dr. Nirmala Upadhyay, and a voucher specimen (LU 2/23560/01) was deposited in the Department of Botany, Lucknow University, Lucknow, India.

Extract Preparation

Leaves were air dried in shade, grinded mechanically and 100g of coarse powder was extracted by using 1,000ml of 95% ethanol in soxhlet. Extract was concentrated to a semi-solid alcohol free material and final extract yield was 8.74% (based on wet material). All the chemicals used were of analytical grades from E. Merk Ltd., West Germany and Sigma-Aldrich Chemicals, USA.

Animals

Male albino rats of Charles Foster strain (120-140g) were used. Animals were maintained on Hind Lever pellets diet (Mumbai, India), housed in polypropylene cages at a temperature of 23 ± 2°C and relative humidity 60 ± 5 with 12 hour each of dark and light cycles. Water was allowed *ad libitum*. The experimental protocols were conducted in accordance with internationally accepted standard guidelines for care and use of laboratory animals.

Experimental Design

The rats were divided in to experimental and control groups of six rats each. Extract was suspended in to 2% gum acacia. Acute toxicity study on behavioral changes was carried up to dose of 2500mg/kg body weight and found to be safe. Extract was fed to experimental group by metal canula at a single dose of 250mg/kg body weight. Control group was fed 2% gum acacia suspension. The blood glucose lowering efficacy was examined in following four experimental models of male albino rats:

(a) Fasted Model: Blood was collected (at 0 hour) from the tail vein of the overnight (18 hours) fasted rats and extract was fed. Again blood samples were collected at 1, 3 and 4 hour interval after feeding extract. Blood glucose concentrations were estimated by Nelson's Somogyi method [9].

(b) Fed Model: Excess amount of pellets were put in the cages on previous evening, so that some pellets are left over in the next morning. Blood was collected before (at 0h) and after administration of the extract at 1, 3 and 4 hour interval for glucose estimation.

(c) Glucose loaded Model: Animals were fasted for 18h and blood was collected (at 0h) for glucose estimation. Now extract was fed and half an hour after this glucose (1.5g/kg b.w. oral) solution was administered and blood samples were collected for glucose estimation at 1/2, 1 and 3 hour interval.

(d) Diabetic Model: Diabetes was induced in rats by injecting streptozotocin (35mg/kg b.w.) dissolved in the chilled citrate-phosphate buffer (*pH* 4.5) through tail. At the moment of injection the rats had fasted for 18 hours. After the streptozotocin injection, all rats were returned to their cages and given free access to food and water. Diabetes was confirmed by checking urine glucose by diastex after 48 hours of STZ-injection [10]. Then the experiments were performed using identical procedure as in the fasted model (a).

Study on Muscle Glycogen

Muscle glycogen content of extract treated and control group of well fed normal rats (each of 5 animals) was estimated at the most effective hour of blood glucose lowering. Rats were sacrificed and portion from muscle tissue of diaphragm was taken out quickly for glycogen estimation [11].

Statistical Analysis of Data

Mean and standard error were determined and 'Student's *t*–test' was performed between experimental and control group. The results were considered statistically significant, if the *p* values were 0.05 or less.

Histological Study of Pancreatic β-cells

Pancreas of anesthetized animals were taken out at the most effective hours of associated blood glucose lowering and fixed in alcoholic Bouin's fluid. Paraffin sections of 10µm thickness were double stained with haematoxylin and eosine, and granulation in β-cells of islets of Langerhans, was compared under microscope [12, 13].

RESULTS

Blood Glucose Levels

It is clear from Table 1 that in fasted group of rats ethanol extract of *C. tamala* (leaves) produced significant ($p<0.01$) lowering of blood glucose after 3

Table 1. Effect of 95% ethanol extract of *Cinnamomum tamala* leaves at a single oral dose of 250mg/kg body weight on blood glucose levels of fasted, fed and streptozotocin-induced diabetic male albino rats

Group	Treatment	Blood glucose level mg/100ml (Mean ±S.E.) at time (hours)				Maximum % of blood glucose lowering from initial value
		0h	1h	3h	4h	
Fasted Model	Control	84.17± 4.91 (6)	82.86± 2.77 (6)	79.40± 2.23 (6)	79.65± 2.76 (6)	5.66% at 3h
	C. tamala Leaves	88.70± 2.73 (6)	75.68± 3.25 (6)	65.83± 2.07[b] (6)	69.28± 0.96[b] (6)	25.78% at 3h
Fed Model	Control	102.95± 1.88 (6)	100.15± 2.17 (6)	102.08± 2.31 (6)	98.89± 1.58 (6)	3.94% at 4h
	C. tamala Leaves	100.49± 1.64 (6)	101.67± 1.07 (6)	92.84± 1.20[b] (6)	99.55± 1.64 (6)	7.61% at 3h
Diabetic Model	Control	198.51± 3.18 (6)	210.39± 6.52 (6)	203.85± 6.58 (6)	205.96± 5.59 (6)	No lowering
	C. tamala Leaves	192.53±7.34 (6)	189.32± 7.40 (6)	179.99± 6.19[a] (6)	162.76± 7.95[b] (6)	15.46% at 4h

Significance between the control and experimental group: [a]$p<0.05$, [b]$p<0.01$
In parenthesis the number of rats used is given.

Table 2. Effect of 95% ethanol extract of *Cinnamomum tamala* leaves at a single oral dose of 250mg/kg body weight on glucose tolerance (1.5g/kg b.w. orally) of male albino rats

Group	Treatment	Blood glucose level mg/100ml (Mean ±S.E.) at time (hours)				Maximum % of blood glucose rise from initial value
		0h	1/2h	1h	3h	
Glucose Loaded Model	Control	75.49± 3.01 (6)	90.74± 5.10 (6)	94.05± 6.04 (6)	92.16± 2.78 (6)	24.58% at 1h
	C. tamala Leaves	72.79± 2.33 (6)	79.53± 1.94 (6)	79.85± 2.67 (6)	76.37± 1.85[c] (6)	9.70% at 1h

Significance between the control and experimental group: [c]$p<0.001$
In parenthesis the number of rats used is given.

and 4 hours of extract treatment as compared to control. Blood glucose lowering calculated in terms of maximum percentage from the initial (0h) value was higher in extract treated rats (25.78% at 3h) than control group (5.66% at 3h).

In fed group of rats, ethanol extract produced significant ($p<0.01$) blood glucose lowering at 3h post-treatment. Extract induced maximum percentage of blood glucose lowering (7.61% at 3h) from initial (0h) value was higher than control (3.94% at 4h).

In diabetic group of rats, extract produced significant lowering at 3 ($p<0.05$) and 4 ($p<0.01$) hours post treatment. Maximum percentage of blood glucose lowering in treated group was 15.46% at 4h and in control group (2% gum acacia) no lowering was observed.

In glucose loaded model (Table 2), *C. tamala* (leaves) extract produced very significant ($p<0.001$) suppression of peak value at 3h post-treatment as compared to respective control. Assessed from the maximum percentage of blood glucose rise, leaves extract was found to possess more blood glucose lowering effect (9.70% rise at 1h) than 2% gum acacia (24.58% rise at 1h).

Muscle Glycogen Content

In fed group of rats leaves extract significantly ($p<0.01$) increased the muscle glycogen content at 3h post-treatment associated with corresponding decrease in blood glucose level. Muscle glycogen content in extract treated group is 651.6 ± 22.45 mg/100gm of tissue and in control group is 484.4 ± 38.22 mg/100gm of tissue.

Histological Examination

Photomicrographs of sections of pancreas of *C. tamala* extract treated fasted (Fig 3B) and fed (Fig 3D) rats clearly showing prominently degranulated β-cells at 3 hours post-treatment associated with maximum blood glucose reduction as compare to their respective control group of untreated rats (Fig 3A and 3C). During examination of histological sections of pancreas in glucose loaded model, marked degranulation in β-cells of islets of Langerhans of extract treated rats (Fig 3F) can be observed at the most effective hour (3h) of blood glucose lowering as compared to control (Fig 3E).

DISCUSSION

In our systematic investigation on the blood glucose lowering efficacy of *C. tamala* we have used four different experimental models of male albino rats to increase the sensitivity of detection, not incorporated in previous studies. In the present study, the hypoglycaemic activity of *C. tamala* leaves extract has been confirmed in normal fasted rats as the onset of its action starts 1 hour after extract feeding, significantly peaked within 3 hours and persists 4 hours of treatment compared to initial. In extract treated fasted rats degranulation in pancreatic β-cells suggests that the enhanced insulin secretion from β-cells is responsible to make possible increase in peripheral utilization of glucose.

Same results explored in the fed model, where extract probably induced insulin release from β-cells is lowered the blood glucose levels by promoting the shunting of glucose particularly towards muscles and increasing muscle glycogen stores most probably mediated through the enhanced process of glycogenesis as compared to control.

Streptozotocin-induced diabetes which resulted from almost complete destruction of the pancreatic β-cells, cause a deficiency or massive reduction in insulin release and procucing hyperglycaemia which insulin only can reverse. In our present study we have observed

Fig 1. *Cinnamomum tamala* (Hamm.) Nees. & Eberm

Fig 2. *Cinnamomum tamala* dried leaves

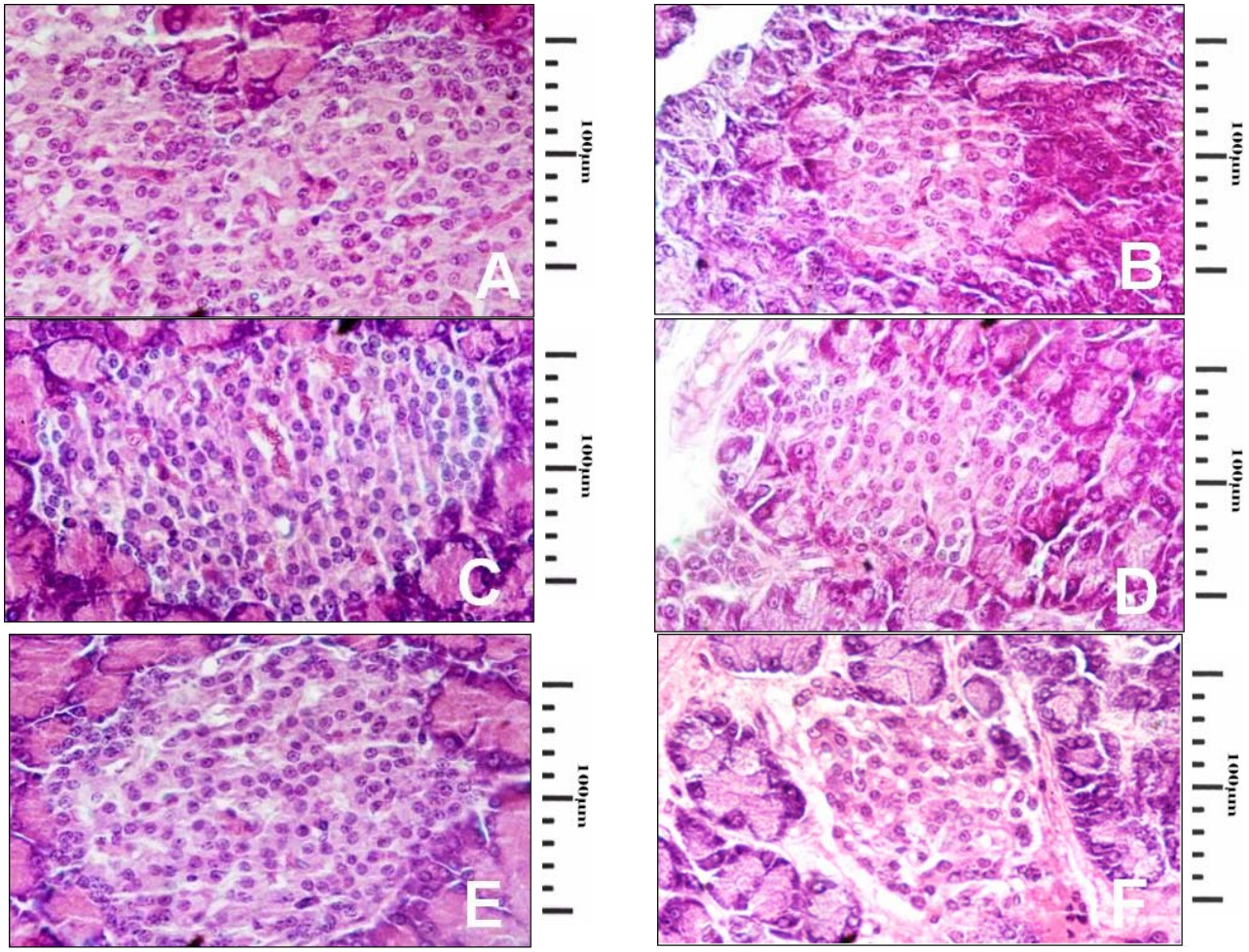

Fig 3(A-F). Microscopic observation of Histological sections of pancreas of *C. tamala* (leaves) extract treated and control (2% gum acacia treated) rats (Magnification-63X).
(A) Section of Fasted rat pancreas, sampled at 3 hour post-2% gum acacia treatment (control), showing normal rich granularity of β-cells of islets of Langerhans.
(B) Section of Fasted rat pancreas, sampled at 3 hour post-*C. tamala* extract treatment associated with maximum blood glucose lowering, showing marked degranulation in β-cells of islets of Langerhans as compared to control (A).
(C) Section of Fed rat pancreas, sampled at 3 hour post-2% gum acacia treatment (control), showing normal rich granularity of β-cells of islets of Langerhans.
(D) Section of Fed rat pancreas, sampled at 3 hour post-*C. tamala* extract treatment associated with maximum blood glucose lowering, showing prominent degranulation in β-cells of islets of Langerhans as compared to control (C).
(E) Section of Glucose loaded rat pancreas, sampled at 3 hour post-2% gum acacia treatment (control), showing normal rich granularity of β-cells of islets of Langerhans.
(F) Section of Glucose loaded rat pancreas, sampled at the most effective hour of blood glucose lowering, *i.e.* 3 hour post-*C. tamala* extract treatment, showing marked degranulation in β-cells of islets of Langerhans as compared to control (E).

that extract of Tejpat leaves can reverse the hyperglycaemic condition in diabetic rats and bring about hypoglycaemic action because blood glucose once lowered by the extract did not increase again throughout experiment as compared to untreated control where the blood glucose level is always remaining above the initials. The possible mechanism of action of the leaves extract may be by promoting the insulin release from the undestroyed β-cells or its action may be insulin like as reported by Chandola *et al* [5].

In glucose loaded model, after glucose feeding to extract treated and control group of fasted rats, the blood glucose concentrations were increased to maximum value at 1 hour, and then returned to almost initial values at 3 hours post-treatment in case of extract treated rats but remained high in control group of rats. Their histological observations suggest that extract could be improving the oral glucose tolerance by increasing the availability of insulin.

CONCLUSION

In conclusion, it may be stated that our observations are suggestive of the fact that the 95% ethanol extract of *C. tamala* leaves possess a hypoglycaemic activity in normal and diabetic male albino rats and can be improve the oral glucose tolerance by promoting the peripheral utilization of glucose and increasing the muscle glycogen store probably induced by stimulating insulin release from β-cells or through its insulin like action.

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