

ANTIDIABETIC AND ANTIOXIDANT ACTIVITIES OF CINNAMOMUM TAMALA LEAF EXTRACTS IN STZ-TREATED DIABETIC RATS

ABSTRACT

The present study evaluated the anti-hyperglycemic activity of the aqueous extracts of *Cinnamomum tamala* (CTLEt) leaves on blood glucose of albino rats. CTLEt was administered at doses of 125 and 250 mg/kg body weight respectively on streptozotocin induced diabetic rats for 3 weeks. Diabetic rats had much reduced body weight than normal rats. Administration of the extracts at the dose of 250 mg/kg body wt. /day resulted in a marked decrease in the levels of fasting blood glucose and urine sugar, with a concomitant increase in body weight. The extract also produced a significant decrease in peroxidation products, viz., thiobarbituric acid reactive substances. Reduced glutathione and glycogen content, which had shown significant decrease following induction of diabetes, were found to be increased in the hepatic tissue of STZ-diabetic rats treated with CTLEt. STZ-diabetic rats treated with CTLEt (250mg/kg) significantly reversed all these changes to near normal. Quantification of antioxidants of the leaves-phenols, ascorbate and carotenoids revealed that *C.tamala* leaves had high antioxidants. These results suggest that CTLEt induce antihyperglycemic as well as antioxidant activities in STZ-diabetic rats.

INTRODUCTION

India has one of the oldest, richest and diverse cultural traditions associated with the use of plants and herbs for human, livestock and plant health. Many of the ingredients of Indian cooking which have been handed down from ages contain medicinal properties. A vast ethnobotanical knowledge exists in India from ancient times. However, very few plants used by locals

Mohammad Chand Jamali & Mukta Sharma

CMJ University, Meghalaya

developing countries like India, taking into account the socio-cultural and economic conditions is urgent.

Diabetes is a deadly disease that affects an estimated 135 million people worldwide and the numbers are increasing in rural and poor populations throughout the world. Diabetes mellitus is a non-communicable disease considered to be one of the five leading causes of death world-wide. In the indigenous Indian system of medicine, good number of plants was mentioned for the cure of diabetes and some of them have been experimentally evaluated and the active principles isolated. However search for new anti-diabetic drugs continues.

Cinnamomum tamala Fr. Nees., belonging to family Lauraceae, is also known as Indian Cassia and the leaves are commonly called as bay leaves. Lauraceae is an economically important family consisting mostly of trees or tree-like shrubs. The genus *Cinnamomum* is represented by about 350 species worldwide. It is native to South-east Asia, some Pacific Islands and Australia, growing mainly in tropical rain forests at varying altitudes. Historically, it is one of the oldest known and used spices.

C. tamala which is an evergreen tree up to 8m in height is also cultivated. Natural habitat is in the tropical and sub-tropical Himalayas at altitudes of 900-2500m. Due to its aroma, the leaves are kept in clothes and also chewed to disguise bad mouth odour. Its dried leaves are used as a common ingredient of Indian cooking. The leaves of this tree have a clove like taste and a faintly pepper like odor. It is also used in Indian system of traditional medicines. Leaves and bark have aromatic, astringent, stimulant and carminative qualities and used in rheumatism, colic, diarrhea, nausea and vomiting. Ancient literature has revealed that in the first century A.D., dried leaves and bark of this plant were prescribed for fever, anemia and body odor. Its seeds were crushed and mixed with honey or sugar and administered to children for dysentery or cough.

The present study focuses specially on the antioxidant and Antidiabetic activities of leaf extracts of *C.tamala* which is used in Indian cooking and which was obtained locally.

MATERIALS AND METHODS

Cinnamomum tamala Fr Nees is a moderate sized evergreen tree 7.5 m in height with dark brown or blackish rough bark and pinkish or reddish brown blaze. *C. tamala* was collected from Dakshin Dinajpur district, West Bengal, India during June, 2006. It was identified by the Botanical Survey of India, Kolkata, India and the voucher specimen (Acc. No. 9490) was deposited to the herbarium of the University of North Bengal.

Preparation of Plant Extract: Leaves of *C.tamala* (500g) were extracted separately with 1.5 L of water by the method of continuous hot extraction at 60°C for 6 h and evaporated. The residual extract was dissolved in water and used in the study. These were designated as CTLEt.

Animals: Male Wister albino rats (180-200g) were obtained from Ghosh Enterprise, Kolkata. The animals were grouped and housed in polypropylene cages and maintained under standard laboratory conditions (temperature 25±2°C) with a 12-h/12-h dark and light cycle. All animals were maintained on a standard laboratory diet and tap water and had free access to food and water. All procedures described were reviewed and approved by the North Bengal University Animals Ethical Committee.

Drugs and Chemicals: All the drugs and biochemical used in this experiment were purchased from the Himedia Laboratory, Mumbai, India and the chemicals were of analytical grade.

Induction of Experimental Diabetes: The animals were allowed to fast for 18 hr and a freshly prepared solution of Streptozotocin (55mg/kg, i.p.) in 0.1M citrate buffer, pH 4.5, was injected intra-peritoneally in the animals (single injection) in a volume of 1ml/kg body wt. After 48 h of STZ administration, rats with moderate diabetes having glycosuria and

hyperglycemia (i.e., with blood glucose of greater than 200 mg/dl) were used in the investigation.

Experimental Design: In the experiment, a total of 40 rats (24 diabetic surviving rats, 16 normal rats) were used. The rats were divided into 5 groups of 8 rats each. Group-I untreated rats receiving distilled water were designated as control and Group-II rats receiving 0.1M citrate buffer (pH 7.5) were also designated as buffer control. Group-III with STZ treatment alone was considered as diabetic control. After 48 h of STZ induction diabetic rats treated with the CTLEt were grouped into Group-IV (125mg/kg body wt.) and Group-V (250mg/kg body wt.). STZ treated diabetic rats were fed with the plant extracts in dist water using intragastric tube twice a day for 20 days. The body weight gain, fasting blood glucose and urine sugar of all the rats were determined at regular intervals during experimental period.

After 20 days, all the rats were fasted overnight and sacrificed by cervical decapitation. Blood was collected in tubes containing sodium fluoride for the estimation of fasting blood glucose. Livers were removed immediately, rinsed in ice chilled normal saline and patted dry and weighed.

Biochemical Analyses

Qualitative Determination of Urine Sugar: Glucose was detected in the urine by the method of Benedict et al. Benedict's reagent (5ml) was taken in a test tube and 8 drops of urine was added to it. Tubes were boiled for 2 min and then cooled slowly. The solutions were filled with greenish/yellow/red or no precipitate depending upon the quantity of glucose present. Greenish precipitate would indicate very small amount of glucose. The solution remained clear where no glucose was there.

Estimation of Fasting Blood Glucose: Fasting blood glucose was quantified by the method of Nelson. Proteins were precipitated by treatment with barium hydroxide and zinc sulphate and removed prior to estimation.

Quantitative Estimation of Glycogen: Glycogen was hydrolyzed to glucose by the method of Raghuramulu et al. and the glucose thus

formed was estimated by Nelson's method. For estimation of glycogen, the liver was taken out rapidly from the animal and the excess blood removed by blotting between folds and filter paper and immediately put into a weight stopper test tube containing 30% KOH and weighed. The amount of alkali was then adjusted to get 2ml per g of liver. The tissue was digested in a boiling water bath for 1 hr. The filtrate was cooled in ice cold water. Two volumes of 95% ethanol were then added and the mixture heated just to boiling. Spurting was avoided. This was left to stand overnight in the cold. The tubes were centrifuged and the precipitate dissolved in 5-10 ml warm water. The glycogen was re-precipitated with 2 volumes of 95% ethanol. The precipitate was centrifuged and washed several times with 60% ethanol. Two ml of 2 N H₂SO₄ per g of initial liver weights was added and hydrolyzed in a boiling water bath for 3-4 h. The solution was neutralized with NaOH using Phenol red as indicator. Volume was noted and filtered. Glucose was determined in that aliquot. The factor 0.93 used to convert glucose to glycogen.

Determination of Thiobarbituric Acid Reactive Sub-Stances

(TBARS): TBARS in tissues was estimated by the method of Ohkawa et al. [9]. After collection of blood samples the rats were killed and livers were excised, rinsed in ice cold normal saline, followed by rinsing with 0.15 M Tris-HCl (pH 7.4), blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation. To 0.5ml tissue homogenate, 0.5ml saline and 1.0ml 10% TCA were added, mixed well and centrifuged at 3000 rpm for 20 min. To 1.0 ml of the protein free supernatant, 0.25 ml of thiobarbituric acid (TBA) reagent was added; the contents were mixed and heated for 1h at 95°C. The tubes were cooled to room temperature under running water and absorbance measured at 532 nm. The levels of lipid peroxides were expressed as moles of thiobarbituric acid reactive substances (TBARS)/mg protein.

Determination of Reduced Glutathione (GSH): GSH in tissues was estimated by the method of Ellman et al.

Table 1: Effect of *C.tamala* extracts on changes in body weight in normal and experimental rats

Group	Treatments	Body weight (kg)			
		1	2	3	4
I	Normal(only vehicle dH O)	173.5±4.7	175.0±4.	188.7±5.	202.5±5.8
		3	83	54	6
II	Citrate buffer	166.0±4.9	167.0±5.	179.5±4.	191.5±3.5
		9	00	50	0
III	STZ(diabetic control)	171.0±1.1	167.6±0.	156.7±0.	147.0±0.5
		5	66	88	7
IV	STZ+CT(125 mg/kg)	172.5±4.5	174.0±5.	182.5±5.	190.0±2.0
		0	00	50	0
V	STZ+CT(250 mg/kg)	173.0±4.0	176.5±6.	183.0±5.	201.0±8.0
		0	50	00	0

Each value represents mean; \pm -SE; CT = *Cinnamomum tamala* leaf extracts
 1= 1st day of treatment; 2= 2 days after STZ treatment; 3 & 4 = 10 & 20 days after administration of extracts Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with normal After killing the rats, livers were excised, rinsed in ice cold normal saline, followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A part of homogenate after precipitating proteins with Trichloroacetic acid (TCA) was used for estimation of glutathione. An aliquot of 50 μ l sample was mixed with 1.7 ml of disodium hydrogen phosphate solution (0.3 M). The final volume of 2 ml was made by adding 250 μ l of DTNB reagent [4 mg, 5, 5-dithiobis (2-nitrobenzoic acid) in 10 ml of 1% (w/v) sodium citrate]. The absorbance of the sample was measured at 412 nm. GSH solution of known concentrations (10-50 μ g) was simultaneously processed to prepare a

standard curve. The amount of GSH in the sample was calculated using the standard curve generated from known GSH.

Statistical Analysis: The collected data were subjected to statistical analysis by standard procedures of Standard Error, CD and Student's *t* test. Correlation and regression were also determined in MS-Excel plots.

RESULTS AND DISCUSSION

Streptozotocin (STZ)-induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents. Streptozotocin (STZ) destroys β -cells of the pancreas and induces hyperglycemia. Oxidative stress resulting from enhanced free radical formation and/or defects in antioxidants defense causes severe tissue damage and may lead to number of diseases like coronary artery disease, atherosclerosis, cancer and diabetes. Increased oxidative stress in streptozotocin diabetic rats has been reported. In the present study, initially, changes in the body weight on the treatment of diabetic and normal rats with CTLEt were determined and these have been demonstrated in Table 1. There was a significant decrease ($p < 0.01$) in the body weight of the diabetic controls compared with the normal controls. Administration of Each value represents as mean \pm SE, $n=8$; CTLEt = *Cinnamomum tamala* leaf extracts; *After 20 days of administration of extracts.

Table 2: Effect of CTLEt on changes in fasting blood glucose in normal and experimental rats

Group	Treatments	Initial	Final*
I	Normal (only vehicle distilled water)	76.11 \pm 1.05	77.75 \pm 2.89
II	Citrate buffer treated	76.85 \pm 0.68	76.85 \pm 1.13
III	Diabetic Control	76.40 \pm 0.81	337.07 \pm 6.36
IV	CTLEt (125mg/kg)	78.20 \pm 1.07	114.26 \pm 0.84
V	CTLEt (250mg/kg)	77.53 \pm 0.29	73.20 \pm 1.86

Values were statistically significant at $p < 0.01$ as compared with diabetic control, $p < 0.01$ as compared with normal.

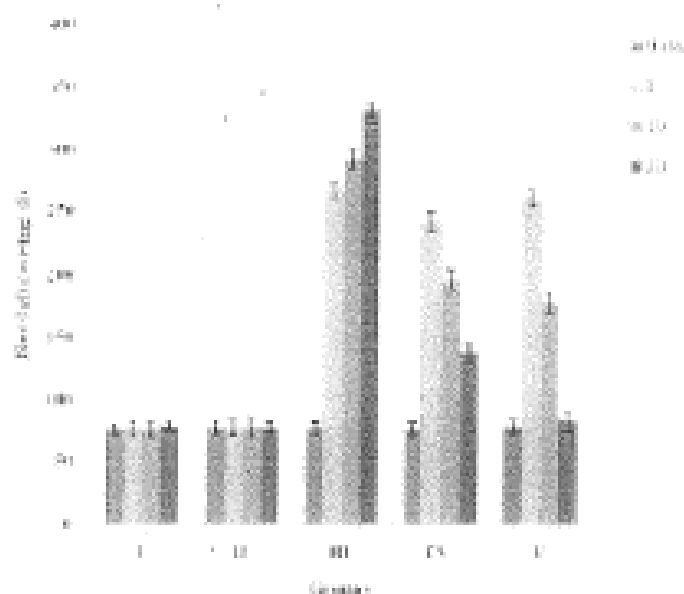


Fig. 1: Blood glucose levels in different groups of treated rats. Group I =control; Group II= Buffer control; Group III= Diabetic control; Group IV and Group V= CTLEt (125 and 250 mg/kg body wt.) treated groups. 0 and 2= days after STZ-treatment; 10 & 20= days after plant extract treatments

CTLEt to diabetic rats (Groups IV and V) increased body weight significantly ($p < 0.01$) which was comparable to the increase in the body weight of normal controls. Changes in the urine sugar of different treated groups revealed that the results of the test were positive (+++) in the diabetic rats and amount of sugar were nil in the CTLEt (250mg/kg) treated groups. Less amounts (+) of sugar was found in the groups receiving lower concentration of the extracts. Decrease in bodyweight due to derangement of metabolic pathways is a common feature in diabetes. Results of the present study support partially the findings of Panache et al. and Perez et al. who also observed significant increase in body weight after treatment with herbal

oral administration of the methanol fraction of *Alicia reticulata* twice daily to the diabetic animals which gained body weight.

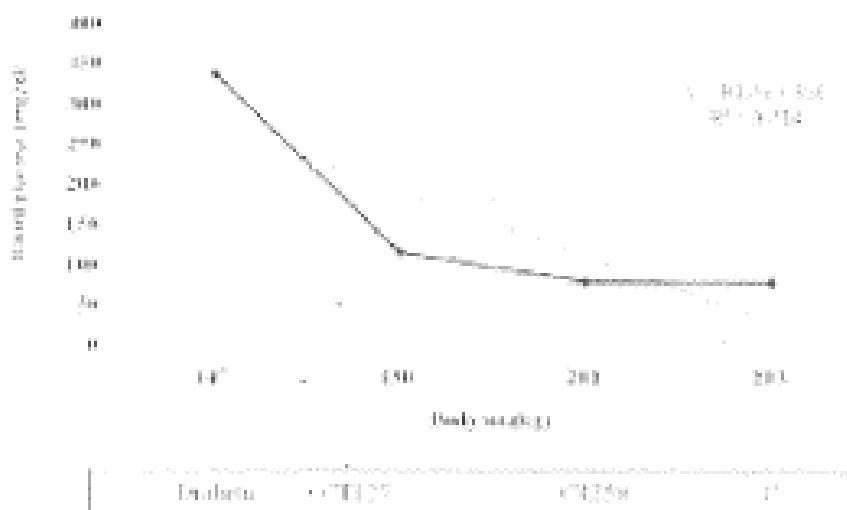


Fig. 2: Correlation and regression between body weight and blood sugar content.

Fasting blood glucose levels in the normal controls rats remained unchanged during the course of the experiment. There was a significant ($p < 0.01$) increase in blood glucose in diabetic rats after two days of STZ administration. Administration of CTLEt (250mg/kg) decreased significantly ($p < 0.01$) the level of fasting blood glucose and brought the sugar level near to normal (Table 2 and Fig. 1). A negative correlation was obtained between body weight and blood glucose content (Fig. 2). Experiments of Venkateswaran and Pari revealed that the administration of *Coccinia indica* leaves' extract in diabetic rats restored the level of blood glucose to near normal levels. Results of other scientists are also in conformity with the present work. The effects of gliclazide and three herbal preparations i.e. neem leaf extract (NLE), *Cathartanthus roseus* leaves' extract and bitter melon fruits' juice significantly decreased blood glucose level [$p < 0.01$] with a highest reduction of 45% by gliclazide. In a study with aqueous extract of

Murraya koenigii leaves, a common spice in Indian cooking, Kesari et al reported that various doses of the extract could lower blood glucose levels in alloxan induced diabetic rabbits.

The level of glycogen decreased significantly ($p < 0.001$) in the STZ-diabetic rats as compared to control. Treatment with the extracts (250mg/kg and 125mg/kg) significantly ($p < 0.001$) increased the glycogen and brought them near to normal level (Table 3). Similarly the concentrations of GSH in tissues in the liver of STZ-diabetic rats were significantly ($p < 0.01$) decreased. Administration of CTLEt (250mg/kg and 125mg/kg) increased the levels of GSH in the liver ($p < 0.01$) during diabetes (Table 4). Diabetes mellitus is associated with a marked decrease in the level of liver glycogen. Pari and Latha showed that GSH level was significantly lower in diabetic rats than in normal rats. Administration of *Cassia auriculata* flower extracts at 0.45g/kg body weight and glibenclamide increased significantly the GSH levels as compared with the levels in diabetic rats. Pari and Latha have further shown that the hepatic and skeletal muscle glycogen content was reduced significantly in diabetic control. Our findings were also in conformity with their study. Murugesh et al also observed a decrease in GSH in the liver during diabetes. The decrease in liver GSH levels represents increased utilization due to oxidative stress.

Table 3: Effect of CTLEt on glycogen in control and diabetic rats

Groups	Treatment	Glycogen (mg/100g)
I	Normal	40.65 ± 2.58
II	Citrate Buffer	41.40 ± 2.77
III	Diabetic Control	21.18 ± 2.66
IV	CTLEt (125mg/kg)	33.41 ± 1.24
V	CTLEt (250mg/kg)	44.05 ± 1.24

Each value represents as mean ± SE, n=8; Values were statistically significant at $p < 0.001$ as compared with diabetic control. CTLEt = *Cinnamomum tamala* leaf extract

Table 4: Effect of CTLEt on reduced glutathione (GSH) in control and diabetic rats

Groups	Treatment	(mM/100g tissue)
I	Control(only vehicle dH O 2	45.33±1.76
II	Citrate buffer control	46.00±1.52
III	Diabetic control	25.66±1.85
IV	CTLEt (125 mg/kg)	35.66±1.20
V	CTLEt(250 mg/kg)	45.00±0.57

Each value represents mean± SE, n=8; Values were statistically significant at $p < 0.001$ as compared with diabetic control

CTLEt = *Cinnamomum tamala* leaf extract

Table 5: Effect of CTLEt on TBARS level in control and diabetic rats

Groups	Treatment	TBARS (mM/100g)
I	Control(only vehicle dH O 2	0.82±0.03
II	Citrate buffer control	0.79±0.05
III	Diabetic control	1.84±0.12
IV	CTLEt (125 mg/kg)	1.16±0.02
V	CTLEt(250 mg/kg)	1.01±0.02

Each value represents as mean± SE, n=8; Values were statistically significant at $p < 0.001$ as compared with diabetic Control

CTLEt = *Cinnamomum tamala* leaf extract

Components	Quantity
Phenols (mg/g dry wt.)	20.83 ±0.11
Ascorbate (mg/g dry wt.)	22.30± 0.21
Carotenoids (mg/g dry wt.)	0.82± 0.04
Antioxidants (mg α -tocopherol acetate equivalent/g dry wt.)	11.27±0.09

Changes in the concentration of TBARS in the liver of STZ-diabetic rats on the treatment with CTLEt are depicted in Table 5. The STZ-diabetic rats showed a significant increase in TBARS (1.84 mM/100g) when compared with normal (d H O-0.81 mM/100g and citrate 2 buffer-0.79 mM/100g) in liver. Thiobarbituric acid reactive substance levels were decreased in the CTLEt treated groups when compared with the normal rats. There was a significant ($p < 0.001$) reduction (1.00 mM/100g) in TBARS in the liver of rats with the treatment of CTLEt when compared with normal rats. Kamalakkannan and Stancy investigated the antidiabetic and antioxidant activity of *Aegle marmelos* in streptozotocin-induced diabetic rats. In their study diabetic rats showed a significant increase in TBARS and hydroperoxides in liver and kidney. Oral administration of *Aegle marmelos* fruit extract maintained the tissue TBARS and hydroperoxides to near normal status which was similar to our findings. Results of the present study also support the findings of Pari and Maheswari. They showed that banana flower extract (BFEt) had an antihyperglycaemic action. The decrease in thiobarbituric acid reactive substances (TBARS) and the increase in reduced glutathione (GSH) clearly showed the antioxidant property of BFEt. A significant decrease in the levels of GSH was observed in CTLEt treated rats when compared with STZ-induced diabetic controls. In the present study, good correlation was obtained between changes in blood sugar due to different treatments and corresponding changes in the other components. Quantification of antioxidants was done in both the leaves.

C.tamala had high antioxidants (Table 6). Results of the present study demonstrate that the leaf extracts of *C.tamala*, commonly used in cooking has an antidiabetic and antioxiadtive activity which has been demonstrated in artificially induced diabetic conditions.

REFERENCES

- Misra, M.K., 1999. Need for conservation of indigenous medicinal knowledge and the herbs. *J. Hum. Ecol.*, 10:403-406.
- Muruges, K., V. Yeligar, D.K. Dash, P. Sengupta, B.C. Maity and T.K. Maity, 2006. Antidiabetic, Antioxidant and Antihyperlipidemic status of *Heliotropium zeylanicum* extract on streptozotocin- induced diabetic rats. *Biol. Pharm. Bull.*, 29: 2202- 2205.
- Grover, J.K., S. Yadav and V. Vats, 2002. Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.*, 81: 81-100.
- Niyonzima, G. and A.J. Vlietinck, 1993. Hypoglycaemic activity of *Spathodea campanulata* stem bark decoction in mice. *Phytother. Res.*, 7: 64-67.
- Siddique, O., Y. Sun, J.C. Lin and Y.W. Chien, 1987. Facilitated transdermal transport of insulin. *J. Pharma. Sci.*, 76: 341-345.
- Benedict, S.R., 1908. A Reagent for the detection of reducing sugars. *J. Biol. Chem.* 5: 485-487.
- Nelson, N., 1944. A photometric adaptation of the Somogyi's method for the determination of glucose. *J. Biol. Chem.*, 153: 357-380.
- Raghuramulu, N., M.K. Madhavan and S. Kalyanasundaram, 2003. A manual of laboratory techniques. National Institute of Nutrition. Ind. Council Med. Res. Hyderabad, pp: 105-106.
- Okhawa, H., N. Oshishi and K. Yag, 1979. thiobarbituric acid reaction. *Anal. Biochem.*, 95: 351-358.
- Ellman, G.L., 1959. Plant antioxidants. *Arch. Biochem. Biophys.*, 82: 70-77.

Diabetogenic action of streptozotocin. Relationship of dose to metabolic response. J. Clin. Invest, 48: 2129-2139.

Palmer, A.M., C.R. Thomas, N. Gopaul, S. Dhir, E.E. Anggard, I Poston and R.M. Tribe, 1998. Dietary antioxidant supplementation reduces lipid peroxidation but impairs vascular function in small mesenteric arteries of the streptozotocin diabetic rats. J Ethnopharmacol., 68: 148-156.

Garg, M.C. and D.D. Bansal, 2000. Protective antioxidant effect of vitamins C and E in streptozotocin induced diabetic rats. Ind. J. Exp Biol., 38: 101-104.

Al-Shamaony, L., S.M. Al-Khazraji and J.I.A. Twaiji, 1994. Hypoglycemic effect of *Artemisia herba alba* L. Effect of a valuable extract on some blood parameters in diabetic animals. J Ethnopharmacol., 43: 167-171.

Ponnachan, P.T.C., C.S. Paulose and K.R. Panikkar, 1993. Effect of leaf extract of *Aegle marmelose* in diabetic rats. Ind. J. Exp. Biol., 31: 345-347.

Perez, C., E. Dominguez, J.M. Ramiro, A. Romero, J.E. Campillo and M.D. Torres, 1998. A study on the glycaemic balance in streptozotocin-diabetic rats treated with an aqueous extract of *Ficus carica* (fig tree) leaves. *Phytother. Res.*, 10:82-83.

Kumara, R.N.K.V.M., R.N. Pathirana and C. Pathirana, 2005. Hypoglycemic activity of the root and stem of *Salacia reticulata* var. *diandra* in alloxan diabetic rats. *Pharma. Biol.*, 43: 219-225.

Venkateswaran, S. and L. Pari, 2002. Effect of *Coccinia indica* on blood glucose, insulin and key hepatic enzymes in experimental diabetes. *Pharma. Biol.*, 40: 165-170.

Bajaj, S. and B.P. Srinivasan, 1998. Investigations into the anti-diabetic activity of *Azadirachta indica*. *Ind. J. Pharmacol.*, 31: 138-141.