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ANTIDIABETIC AND AMELIORATIVE POTENTIAL OF *FICUS BENGALENSIS* BARK EXTRACT IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Mahalingam Gayathri and Krishnan Kannabiran

School of Biotechnology, Chemical and Biomedical Engineering, VIT University, Vellore-632 014, Tamil Nadu, India.

ABSTRACT

The aim of the present study was to evaluate the antidiabetic and ameliorative potential of aqueous extract of Ficus bengalensis bark in streptozotocin induced diabetic rats. The effect of oral administration of aqueous extract of F. bengalensis bark on blood glucose, serum electrolytes, serum glycolytic enzymes, liver microsomal protein, hepatic cytochrome P-450 dependent monooxygenase enzymes and lipid peroxidation in liver and kidney of streptozotocin -induced diabetic rats was studied. Oral administration of Ficus bengalensis to fed, fasted and glucose loaded diabetic rats significantly [F > 0.05 (ANOVA) and P< 0.05 (DMRT)] decreased the blood glucose level at 5 hrs and restored the levels of serum electrolytes, glycolytic enzymes and hepatic cytochrome P-450 dependent enzyme systems and decreased the formation of liver and kidney lipid peroxides at the end of 12 weeks. Further, the aqueous extract of Ficus bengalensis at a dose of 500mg/kg/day exhibits significant antidiabetic and ameliorative activity as evidenced by histological studies in normal and Ficus bengalensis treated streptozotocin induced diabetic rats. On the basis of our findings, it could be used as an antidiabetic and ameliorative agent for better management of diabetes mellitus.

KEY WORDS

Ficus bengalensis, Antidiabetic activity, Ameliorative potential, Cytochrome P-450 dependent monooxygenase enzymes, Lipid peroxidation

INTRODUCTION

Diabetes mellitus (DM) is one of the major metabolic disorders currently associated with considerable morbidity, mortality and several long term complications in the affected individuals. Hyperglycemia is caused either by insufficient insulin secretion or insulin resistance. The percentage of people affected by DM was rapidly rising in India. At present more than 40 million people are affected in India alone which represent nearly 20% of total diabetes population worldwide. The control of blood glucose in diabetic patients was achieved mainly by the use of oral hypoglycemic/antihyperglycemic agents and insulin. However, all these treatments have limited efficacy and have been reported to be associated with undesirable side effects (1-3). In order to overcome the side effects associated with diabetes, interest has been shifted to use of other alternative medicine. Traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of diabetes mellitus. Medicinal plants are continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals (4). Traditional medicine provides better health coverage for 80% of the world population, especially in the developing countries (5).

Ficus bengalensis (Banyan tree), is a large tree with aerial roots. It grows wild in lower Himalayas and is found all over India. Different parts of the tree have been found to possess medicinal properties: leaves are good for ulcers, aerial roots are useful in treating gonorrhea, seeds and fruits are used as cooling agent and tonic as well (6). A water extract of bark of *Ficus bengalensis* (FB) plant has been shown to possess a hypoglycemic effect by different groups of workers (7-9). The water extract of FB bark has been reported to possess

Address for Correspondence :

Dr. K. Kannabiran
Biomolecules and Genetics division,
School of Biotechnology,
Chemical and Biomedical Engineering,
VIT University, Vellore-632014, Tamil Nadu, India.
Tel: +91-0416-2202473
E-mail: kkb_67@yahoo.com

hypocholesterolaemic and hypolipidaemic effects (10). The antioxidant activity of aqueous extract of FB has been reported in hypercholesterolaemic rabbits (11). Three ketones were isolated from the stem bark of FB, they are 20-tetratriacontene-2-one, 6-heptatriacontene-10-one, pentatriacontan-5-one and two other compounds, beta-sitosterol-alpha-D-glucose and meso-inositol have also been isolated (12). A dimethoxy derivative of leucocynidin, 3-O-beta-D-galactosyl cellobioside was also isolated and its antidiabetic activity has been demonstrated (13). A glycoside of leucopelargonidin was also isolated from the bark of *Ficus bengalensis* and its antidiabetic effects have been reported (14). In the present study, the hypoglycemic, antiperoxidative and ameliorative potential of aqueous extract of FB bark was investigated.

MATERIALS AND METHODS

Male albino rats (Wistar strain, weighing 150-200g) were purchased from Tamil Nadu Veterinary and Animal Sciences University, Madhawaram, Chennai and housed under standard husbandry conditions (30° C + 2°C, 60 –70 % relative humidity and 12h : 12h day-night cycle) and allowed standard pelleted rat feed and water *ad libitum*. Animal experiments were designed and conducted in accordance with the guidelines of Institutional Animal Ethical Committee (IAEC).

FB bark was collected from the Morappur forest area in the month of January 2005, Dharmapuri District, Tamil Nadu. The plants were authenticated and voucher specimen was submitted to the Forest Department (FDSC 201). FB bark was washed with distilled water, shade dried, powdered and stored in an air- tight container separately for further use. The bark of FB (100g) was cut in to small slices, powdered and its juice was obtained with a Turmix electric extractor with 500ml of sterile distilled water. The juice was filtered and the residue was removed. The extract was concentrated under vacuum to get solid yield and freeze dried and the yield was calculated.

Diabetes was induced experimentally in rats by single intraperitoneal injection of freshly prepared solution of Streptozotocin (STZ) (Sigma, USA) at a dose of 35mg/kg bodyweight in 0.1M cold citrate buffer, pH 4.5. After 72 h, blood was collected from the tail vein under ether anesthesia with aseptic precautions and blood glucose levels were determined using Autoanalyser Microlab 2000 (Hamilton). Animals were considered to be diabetic if the blood glucose values were always above 250mg/dl and those animals alone were used for the study. Diabetes was developed and stabilized in STZ treated rats over a period of 7 days (15). Control rats were given citrate buffer (pH 4.5) alone. Hypoglycemic activity of the aqueous extract of FB bark was assessed by feeding the extract (500 mg/kg body weight / day) to diabetic control, non-diabetic and STZ- induced diabetic rats and the animals were followed up to 5 hrs to check the time required for the test extract to produce a peak hypoglycemic activity.

Preliminary studies were carried out to determine the time necessary to produce peak hypoglycemic activity after oral administration of plant extract (100, 300 and 500 mg/kg/ day) to a group of 6 rats. Animals were divided in to six groups of six animals each. Group I served as a control rats, group II was STZ- treated surviving diabetic rats, group III served as a positive control and received tolbutamide (100 mg/kg bw / day), group IV- fasted rats; non-diabetic and diabetic rats were fasted for 12 hrs and treated with aqueous extract 500 mg/kg body weight /day by oral intubation method. Group V fed rats; non-diabetic and diabetic rats were provided with unlimited quantity of rat feed and treated with aqueous extract 500 mg/ kg body weight /day by oral intubation method. Group VI glucose loaded model; non-diabetic and diabetic rats were fasted for 12 hrs and treated with aqueous extract 500 mg/kg body weight /day, followed by 10% glucose solution (1.5 g/ kg bw) by oral intubation method. Rats were followed up to 5 hrs, blood glucose levels were estimated at the end of 1hr, 3hr and 5 hr.

Six parallel groups of rats were treated with FB bark aqueous extract (500 mg/kg body weight /day) for 12 weeks. At the end of 12 weeks rats were sacrificed, blood samples, liver and kidney tissues were collected to carry out biochemical and histological studies.

Fasting plasma glucose was estimated by glucose oxidase method (16). The serum was assayed for electrolytes including sodium, potassium and calcium by using flame photometry. Serum marker enzymes, glycogen synthase (17), glucokinase (18), lactate dehydrogenase (19), succinate dehygrogenase (20) and malate dehydrogenase (21) and Liver microsomal monooxygenase enzymes, 7- ethoxyresorufin-O-deethylase (EROD), 7-pentoresorufin-O-depentylase (PROD) and p-nitrophenol hydroxylase (PNPH) (22) were estimated in both control and FB treated rats. Liver and kidney tissue lipid peroxidation was measured by measuring the levels of MDA (23) and hydroperoxides (24).

Statistical analysis was performed using SPSS software package, version 9.05. Experimental results were analyzed by one way analysis of variance (ANOVA) followed by Duncan' multiple range test (DMRT). All the results were expressed as

mean ±SD for six rats in each group *p*-Values <0.05 were considered as significant.

RESULTS

The yield of aqueous extract FB bark was found to be 4.0 % (w/v). STZ treatment increased blood glucose levels significantly ($F > 0.05$; $p < 0.001$) in experimental rats when compared to control rats. Administration of FB bark aqueous extract (500 mg/kg body weight / day) decreased the blood glucose levels significantly ($F > 0.05$; $p < 0.001$) at 5 hrs in STZ – induced diabetic fasted, fed and glucose loaded rats (Table 1). The hypoglycemic activity was equivalent to that of tolbutamide (100 mg / kg body weight / day) treated positive control rats.

Table 1: Effect of *F.bengalensis* on plasma glucose levels in normal and streptozotocin induced diabetic rats

Groups	Experimental sub groups	1hr (mg/dL)	3hr (mg/dL)	5hr (mg/dL)
Normal	-	67±2.09	65±2.6	63±3.0
Diabetic control	-	278±2.28*	273±5.3*	270±5.2*
Diabetic + Tolbutamide	-	195±2.98*	182±2.96*	174±2.52*
Diabetic + *F.bengalensis* (fasted model)	A	68±2.48	65±2.45	64±2.98
	B	172±1.23*	170±2.61*	169±2.65*
Diabetic + *F.bengalensis* (Fed model)	A	89±2.36	71±2.15	69±1.84
	B	196±2.56*	180±2.45*	168±1.54*
Diabetic + *F.bengalensis* (Glucose loaded model)	A	145±1.52	134±2.01	110±1.65
	B	225±1.84*	195±1.95*	173±2.03*

A – Non-diabetic rats ; B – Diabetic rats
Each value is mean ± SD for six rats in each group
*$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to control.

Table 2 presents the levels of glycolytic enzymes in normal and diabetic rats. The metabolic enzymes of glucose was significantly decreased ($F > 0.05$; $p < 0.001$) in STZ -induced diabetic rats when compared to control rats. Oral administration of FB bark aqueous extract (500 mg/kg body weight / day) to STZ -induced diabetic rats brought back the levels of glycolytic enzymes significantly ($F > 0.05$; $p < 0.001$) to near normal levels.

Table 2 : Effect of *F. bengalensis* extract on serum marker enzymes GS, GK, LDH, SD and MD in normal and streptozotocin induced diabetic rats

Groups	GS (U/L)	GK (U/L)	LDH (U/L)	SD (U/L)	MD (U/L)
Normal	8.07±1.25	9.07±2.52	92.74±2.13	4.84±125	5.85±1.42
Diabetic control	5.55±1.36*	4.12±2.31*	60.84±1.54*	3.14±1.45*	1.87±1.32*
Diabetic + Tolbutamide	6.17±1.58	8.65±1.95	89.40±1.45	6.74±1.28	4.32±1.62
Diabetic + *F.bengalensis* (500mg/kg)	7.40±1.65 [a]	8.36±1.54 [a]	89.45±1.45 [a]	6.75±1.42 [a]	3.97±2.36 [a]

Each value is mean ± SD for six rats in each group
*$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to control
[a]$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to diabetic control.

FB bark aqueous extract (500 mg/kg body weight / day) restored the microsomal protein concentration and cytochrome P-450 dependent monooxygenase enzymes EROD, PROD and PNPH in STZ- induced diabetic rats to near normal level (Table 3). The levels of liver and kidney lipid peroxidation

Table 3: Effect of *Ficus bengalensis* on liver microsomal protein, monooxygenase activity in normal and streptozotocin induced diabetic rats

Groups	Microsomal protein (mg/g organ wt)	Monooxygenase activity (nmol product/min/mg protein)		
		EROD	PROD	PNPH
Normal	12.17±0.98	0.22±0.01	0.25±0.02	0.95±0.11
Diabetic control	13.9±0.75*	0.42±0.02*	0.35±0.03*	1.00±0.12*
Diabetic + Tolbutamide	12.9±0.98	0.22±0.03	0.25±0.01	0.96±0.13
Diabetic + *F.bengalensis* (500mg/kg)	12.0±1.8 [a]	0.23±0.03 [a]	0.24±0.03 [a]	0.93±0.16 [a]

Each value is mean ± SD for six rats in each group
*$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to control
[a]$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to diabetic control.

including hydroperoxides and malondialdehyde were significantly ($F > 0.05$; $p < 0.001$) decreased to near normal levels in STZ- induced diabetic rats after treatment with FB bark aqueous extract (500 mg/kg body weight / day) (Table 4).

FB bark aqueous extract (500 mg/kg body weight / day) decreased the levels of serum electrolytes significantly ($F > 0.05$; $p < 0.001$) in STZ -induced diabetic rats (Table 5).

Table 4 : Effect of *F. bengalensis* extract on liver and kidney TBARS, hydroperoxides and malondialdehyde in normal and streptozotocin induced diabetic rats

Groups	TBARS (mM/mg)		Hydroperoxides (n mol/ 100g tissue)		Tissue MDA (n mol/g wet weight)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	0.68±0.21	1.53±0.08	70.64±2.10	55.03±2.09	295.4±1.69	291.23±1.02
Diabetic control	1.45±0.01*	2.64±0.36*	115.57±1.4*	79.00±1.01*	365.3±1.20*	365.23±1.65*
Diabetic + Tolbutamide	1.12±1.01	1.91±0.27	84.13±1.32	65.13±1.11	345.65±1.02	300.95±1.24
Diabetic + *F.bengalensis* (500mg/kg)	1.01±0.52 [a]	1.65±0.58 [a]	77.12±1.12 [a]	56.85±1.33 [a]	304.12±1.47 [a]	271.32±1.47[a]

Each value is mean ± SD for six rats in each group

*$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to control; [a]$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to diabetic control.

Histological examination of pancreas of the STZ -induced diabetic rats showed significant changes in the morphology of pancreatic cells including mild swelling and inflammation. Oral administration of FB bark aqueous extract (500 mg/kg body weight /day) reduced the inflammation and swelling in pancreatic tissue (Fig.1).

Table 5: Effect of *F.bengalensis* extract on serum electrolytes in normal and streptozotocin induced diabetic rats

Groups	Na^+ (mEq/L)	K^+ (mEq/L)	Ca^{2+} (mEq/L)
Normal	145.00±5.90	6.80±1.25	7.38±0.15
Diabetic control	162.00±2.36*	7.90±0.23*	9.10±0.23*
Diabetic + Tolbutamide	143.12±2.12	5.13±0.62	7.91±0.56
Diabetic + *F.bengalensis* (500mg/kg)	136.56±1.25 [a]	4.23±0.47 [a]	6.89±0.65 [a]

Each value is mean ± SD for six rats in each group

* $F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to control;

[a]$F > 0.05$ (ANOVA) and $P < 0.05$ (DMRT) as compared to diabetic control.

DISCUSSION

The present study indicates the hypoglycemic, antiperoxidative and ameliorative potential of FB bark aqueous extract on STZ –induced diabetic rats. Our observations are in well agreement with the reports by several workers that STZ-induced diabetes mellitus and insulin deficiency leads to increased blood glucose (25). It has been reported that STZ at lower doses (60 mg/kg) produces partial destruction of pancreatic β-cells with permanent diabetes condition (26) and there may be more possibility of many surviving β-cells (27). Since a much low dose of STZ was chosen for this study, there may be many surviving β-cells, capable of undergoing regeneration. Administration of aqueous extract of FB bark (500 mg/kg bw per day) decreased the elevated blood glucose level within 5hrs and may be due to the insulin secretogogue effect of the active compound, leucopelargonin (27) present in the extract and prolonged administration may stimulate the β-cells of islets of Langerhans to produce insulin. The antihyperglycemic effect of aqueous extract of FB bark was compared with tolbutamide, a standard hypoglycemic drug. Tolbutamide has long been used to treat diabetes, to stimulate insulin secretion from the pancreatic β-cells. From the results, it appears that still insulin

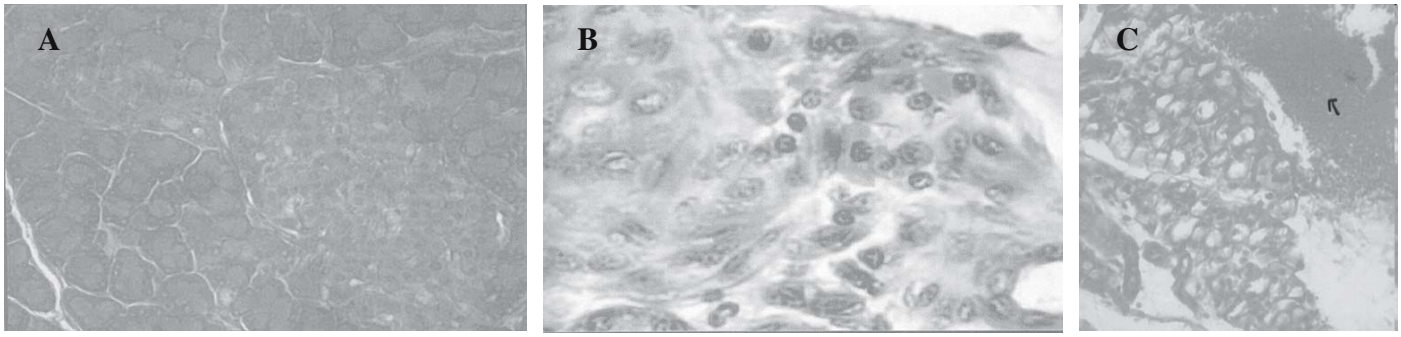

Figure 1: Cross section of pancreas stained with haematoxlin viewed under microscope with 100 X magnification. (A) Showing normal morphology in the control rats. (B) Showing severe swelling and inflammation in cells of the STZ induced diabetic rats. (C) Showing normal architecture and morphology of cells in the FB treated rats.

producing β-cells are functioning in STZ treated diabetic rats and stimulation of insulin release could be responsible for the most of the observed metabolic activities. Further the observed blood glucose- lowering effect in fasted normal and STZ induced diabetic rats could possibly be due to the increased peripheral glucose utilization. A number of other plants have also been shown to exert hypoglycemic activity through stimulation of insulin release (28, 29).

It has been reported that chemically (STZ) induced diabetes produces partial or total deficiency of insulin that results in decrease in the concentration of glycolytic enzymes (30). Insulin has been shown to be potentiator of hexokinse/ glucokinase (31). The decreased levels of glycogen synthase, glucokinase, lactate dehydrogenase, succinate dehydrogenase and malate dehydrogenase may be due to decreased insulin level in diabetic rats. Restoration of the concentration of glycolytic enzymes after oral administration aqueous FB bark extract might be due to its normoglycemic activity. The STZ –induced reduction in the activities of carbohydrate metabolizing enzymes, such as hexokinase, glucose -6-phosphate dehydrogenase and glycogen synthase has been reported to be normalized by the administration of ethanolic extract of *Murraya koenigii, Mentha piperitae, Ocimum sanctum* and *Aegle marmelos* (32).

Chemically induced diabetes has been shown to induce polymorphic alterations on the metabolic activities of cytochrome P-450 dependent monooxygenase enzyme system (33). Type -1 diabetes mellitus induced by long administration of STZ has been shown to be associated with significant modulation of rat hepatic cytochrome P 450 enzyme activities (34). In our study, the elevated concentrations of cytochrome P-450 dependent monooxygenase enzyme system, such as EROD, PROD and PNPH may be due to hepatocellular damage caused by oxygen free radicals. Oral administration of the aqueous extract restored the concentration of hepatic phase I drug- metabolizing enzymes in STZ- induced diabetic rats.

The involvement of free radicals in the genesis of diabetes mellitus and their role in the induction of lipid peroxidation during diabetes has been reported by several workers (35, 36). It has been reported that in diabetes mellitus, oxygen free radicals are generated by stimulating H_2O_2 *in-vitro*, as well as *in- vivo* and in the pancreatic β- cells (37). In our study, the increased tissue malondialdehyde and hydroperoxides in liver and kidney of STZ- induced diabetic rats served as an index of elevated lipid peroxidation in diabetic condition. The increase in lipid peroxidation indicates an increased oxidative stress as a result of excessive generation of free radicals. Administration of FB bark extract 500 mg/kg bw per day for a period of 12 weeks decreased the lipid peroxidation index significantly. The reduction in lipid peroxidation can be attributed to the antioxidant activity of various phytochemicals present in the FB bark aqueous extract. Further, these results suggest that the major function of the extract is to protect vital tissues such as liver, kidney, pancreas and brain from damage and thereby reducing the after effects of diabetes.

It has been reported that elevation of oxidative stress results in depletion of cellular antioxidant scavenger systems and increased free radical –mediated tissue damage by a series of chemical reactions. Hyperglycemia induced vascular damage have already been reported (38). Increased glucose oxidation in the presence of transition metals can cause membrane damage by peroxidation of membrane lipid and protein glycation (39). This could be the reason for the altered flux in electrolyte balance, resulting in elevated extracellular concentration of sodium, potassium and calcium in STZ - induced diabetic rats. Administration of aqueous extract of the FB bark reduced the LPO index and restored the antioxidant status and this could be the possible reason for the restoration of extra cellular electrolyte concentration. FB bark aqueous extract has been shown to possess significant antioxidant effect on cholesterol induced lipid peroxidation (11).

The ameliorative role of FB bark aqueous extract was evidenced by both the observed histological changes, normalization of hepatic phase I drug metabolizing Liver Cytochrome P-450 dependent (CYP) enzymes and reduction in lipid peroxidation index in STZ- induced diabetic rats.

Thus our findings supports the long term use of FB bark aqueous extract at a dose of 500 mg/kg bw per day for better control of blood glucose and restoration of diabetes associated changes. In addition, it also possesses ameliorative and free radical scavenging activity for better control over secondary diabetic complications associated pathogenesis. However, further pharmacological and biochemical investigations are needed to find out the antidiabetic and ameliorative action of the constituents including those studied by Augusti et al (27, 40) and to elucidate their structure and mechanism of action.

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