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Antidiabetic and enzymatic antioxidant properties from methanol extract of *Ficus talboti* bark on diabetic rats induced by streptozotocin

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ABSTRACT

Objective: To explore scientifically, the type-I anti-diabetic potential of *Ficus talboti* bark (FTB). **Methods:** The HPLC analysis was carried out to identify the phenolic compounds. Effect of two doses of methanol extract of FTB (100 mg/kg and 200 mg/ kg body wt.) was orally administered to STZ (Streptozotocin) induced diabetic rats for 21 days. The various parameters were studied including body weight, fasting blood glucose levels, plasma insulin, lipid profile, glycogen content, total protein, serum enzymes levels, and antioxidant activities in normal, treated and diabetic rats. Histochemical analysis of liver and pancreas were also carried out in normal, treated and diabetic rats. **Results:** The HPLC analysis showed the presence of antidiabetic responsible compounds of Rutin, Quercetin and Kaemfeorl. The treatment group with the extract at two dose levels showed a significant increase in the liver, muscle glycogen and serum insulin level and a significant decrease in fasting blood glucose and serum marker enzyme levels. The total cholesterol and serum triglycerides levels were also significantly reduced and the high density lipoprotein and plasma enzymes level was significantly increased upon treatment with the FTB methanol extract. Histochemical study of pancreas also confirmed the biochemical findings. Acute toxicity studies revealed the non-toxic nature of the FTB methanol extract. **Conclusion:** The results of the experiments presented here suggest that methanol extract of FTB exerts significant antidiabetic and antioxidant effect in STZ induced diabetic rats.

1. Introduction

Diabetes mellitus is the name given to a group of disorders with different etiologies. It is characterized by disarrangements in carbohydrates, proteins and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action[1]. Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder caused by destruction of insulin producing β-cells when auto aggressive T-lymphocytes infiltrate the pancreas. This leads to hypoinsulinaemia and thus hyperglycemia. Hyperglycemic condition causes increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy. The currently available antidiabetic agents including sulfonylureas, biguanide, thiazolidinedione and a-glycosidase inhibitors are widely used to control the hyperglycemia and hyperlipidemia, but these drugs fail to significantly alter the course of diabetic complications and have limited use because of undesirable side effects and high rates of secondary failure[2]. Thus, it is essential to look for more effective antidiabetic agents with fewer side effects. Traditional medicinal plants having antidiabetic properties can provide a useful source for the development of safer and effective oral hypoglycaemic agents. The hypoglycaemic activity of a number of plants and plant products have been evaluated and confirmed in animal models as well as in human beings[3]. Currently, the antidiabetic drugs in use for

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long term therapy are found to be associated with various toxicities owing to which the developmental process in antidiabetic drug discovery has shifted its focus to natural plant sources having minimal side effects[4,5]. The present work was undertaken to explore the antidiabetic potential of a plant, Ficus talboti bark (FTB) in type I diabetic animals.

Ficus talboti (*F. talboti*) King belongs to the family Moraceae. It is a wild, infrequent and an endemic to India. It is a large evergreen tree without aerial roots. The ethnobotanical views on *F. talboti*, suggest that aerial parts were used in herbal based drugs in India for diabetic disease[6]. Recently we have reported *F. talboti* fruits have high potential of antioxidant activity and nutritional capacity[7]. *F. talboti* bark possesses good *in-vitro* antioxidant and anti-inflammatory activity[8]. The barks are used for various purposes: as an astringent medicine, for cooling agent, haemostatic, laxative, in improving complexion, in cleaning vagina and useful in *pitta* and *kapha*. It is also widely used in the treatment of skin diseases, ulcer and soreness in the mouth[9,10]. Bark powder is useful in curing venereal diseases, diarrhoea, haemorrhages and leprosy[11]. In our preliminary studies, among petroleum ether, chloroform, acetone, methanol and hot water extracts of the bark, only the methanol extract showed a significant blood glucose lowering effect. However as there are no reports on the antidiabetc activity of *F. talboti* bark, hence the present study was aimed at assessing these in STZ (Streptozotocin) induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Streptozotocin and Glibenclamide were purchased from SIGMA – Aldrich, Mumbai. All other chemicals and reagents used were of analytical grade.

2.2. Plant material and extraction

The fresh plant bark was collected in the month of October 2009, from local areas of Madurai district, Tamil Nadu, India. The taxonomic identity of the plant was confirmed by Dr. A. Rajendran and Voucher specimen was deposited at Botany Department Herbarium, Bharathiar University Coimbatore, Tamil Nadu. The collected bark was washed under running tap water to remove the surface pollutants. The plant materials were air dried under shade. The dried samples were powdered and extracted with Soxhlet apparatus by successive solvent extraction method. Finally the extracted samples were evaporated by using rotator evaporator (Yamato, Japan). The dried extracts were used for further studies.

2.3. High-performance liquid chromatography (HPLC) analysis

Quantification and identification of phytochemicals were carried out on a high performance liquid chromatographic system by following the method of Ye *et al.*[12]. For quantification, the methanol extract was dissolved in 0.5 mL of 100% methanol at a concentration of 30 mg/mL. Waters 515 HPLC (Waters Corp., Milford, MA, U.S.A.) equipped with a photodiode array detector (PDA) (Model: Waters 2998, Waters Corp.) was used for separation. Chromatographic separation was performed in the C18 column; 250 mm×4.6 mm; 5 μm. About 20 μL of sample was injected in to the column by the auto sampler. The samples were eluted through the column with a gradient mobile phase consisting of A (0.2% (v/v) acetic acid in water) and B (0.2% (v/v) acetic acid in methanol) with a flow rate of 0.5 mL/min. Prior to injection into the column, a 20 min linear gradient was programmed as follows: 0–4 min B (30%); 4–10 min B (50%); 10–20 min B (80%). The samples were monitored by Detector at 254 nm at flow rate of 1 mL/min at 29 ℃ temperature. Gallic acids, Caffeic acid, Rutin, Ellagic acid, Quercetin and Kaemfeorl were used for the reference. The quantity of compound was calculated by following formula.

$$\text{Sample Recovery Percent} = \frac{\text{Sample peak are} \times \text{weight of standard}}{\text{Standard peak area} \times \text{weight of sample}} \times \text{Purity}$$

2.4. Experimental animals

Healthy adult male Wistar rats (150–200 g), in-house bred at the Animal House of Kovai Medical Centre Hospital College of Pharmacy, Coimbatore, Tamil Nadu, India were used for the study. Rats were housed in polypropylene cages lined with husk in standard environmental conditions [temperature (25±2) ℃, relative humidity (55±10)% and 12:12 light:dark cycle]. The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee and was cleared by same before beginning of the experiment (No. KMCRET/M.Sc./4/2010-11).

2.5. Acute oral toxicity study

Acute oral toxicity of FTB was performed on male Wister rats, according to OECD Guideline 423. Two groups of three rats each were used for the study. Group I served as control and received distilled water. Group II received single oral dose of FTB (2 000 mg/kg). The animals were observed for gross behavioral, neurological, autonomic and toxic effects at short intervals of time for 24 h and then daily for

14 days. Food consumption was monitored daily and body weights were recorded weekly. On the 14th day, animals were sacrificed and all the organs were taken for gross pathological examination.

2.6. Experimental design

Antidiabetic activity of FTB was assessed in normal, glucoseloaded hyperglycemic and streptozotocin-induced diabetic rats. In all studies, the animals were fasted overnight for 16 h with free access to water throughout the duration of the experiment.

2.7. Evaluation of extract on normal healthy rats

This study was carried out by the method of Kar *et al*. [13]. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn from the retro-orbital plexus of the eye under mild ether anesthesia. Serum was separated by centrifugation and glucose was estimated. The animals were then randomly divided into three groups of six animals each. Group I served as control and received distilled water. Groups II and III received FTB orally at the dose of 100 mg/kg and 200 mg/kg. Reducing blood glucose levels were determined in 1, 2, 3 and 4 h following the treatment.

2.8. Evaluation of extract in oral glucose tolerance test

Prakasam *et al*. [14] method was followed for oral glucose tolerance test after healthy rats were divided into four groups of six animals each: Group I served as control received only vehicle (distilled water) and Groups II and III received FTB orally at the dose level of 100 mg/kg and 200 mg/kg, respectively. All the animals were given glucose (2 g/kg) 60 min after dosing. Blood samples were collected from the retro-orbital plexus of the eye just prior to (0 h) and at 30, 60, 90 and 120 min after the glucose loading, and blood glucose levels were estimated.

2.9. Evaluation of extract in streptozotocin-induced diabetic rats

Experimental diabetes was induced by single intraperitoneal injection of 55 mg/kg of streptozotocin (STZ), freshly dissolved in cold citrate buffer, pH 4.5. Control animals received only citrate buffer. Animals were examined after 5 days of STZ injection; fasting animals with blood glucose level above 250 mg/dL were considered as diabetic and included in the study. The animals were randomly assigned into six groups of five animals each and received the following treatments: Group I: Normal control + distilled water; Group II: Diabetic + distilled water; Group III: Diabetic + FTB (100 mg/kg); Group IV: Diabetic + FTB (200 mg/kg) and Group V: Diabetic + glibenclamide (10 mg/kg). The freshly prepared solutions were orally administered daily for 21 days. Body weights and blood glucose level analysis were done weekly on overnight fasted animals. At the end of the experimental period, the animals were fasted an overnight and blood was collected for various biochemical estimations. The animals were sacrificed by cervical decapitation. Organs like liver, pancreas and skeletal muscle were dissected out, immediately rinsed in ice cold saline and stored for further biochemical estimations [15].

2.10. Biochemical analysis

Serum glucose analysis was done by GOD-POD method using Glucose Estimation Kit (Erba Diagnostics, India). Other serum estimations were done spectrophotometrically using standard kits available which included serum insulin (RIA kit provided by BRIT, BARC, India), serum triglycerides (GPO-Trinder method, Erba Diagnostics), and serum total cholesterol (CHOP-PAP method, Erba Diagnostics). Glycogen was estimated in liver and skeletal muscle by the method of Good *et al*. [16] *In vivo* lipid peroxidation, expressed as TBARS (Thiobarbituric acid reactive substances) was estimated in the pancreatic tissue homogenate according to the method of Ohkawa *et al*. [17] SGOT (Serum glutamic oxaloacetic transaminase) and SGPT (Serum glutamic pyruvic transaminase) activities were determined by the method of Reitman and Frankel[18]. The optical Density (OD) was read at 540 nm.Activity of serum ALP (Alkaline phosphatase) was determined by the method of Kind and King[19]. Total Protein concentration was estimated using the BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Rockford, USA). Glutathione peroxidase (GPx) Activity was measured by the method described by Rotruck *et al*. [20]. Catalase activity was studied by Sinha[21]. The activity of superoxide dismutase (SOD) was assayed by the method of Kakkar *et al*. [22].

2.11. Histopathological Studies

The animals were sacrificed and all the organs were examined for absolute and relative organ weights and gross pathological lesions. Paraffin sections of liver and pancreas tissues were taken and stained with haematoxylin and eosine to observe histopathological changes.

2.12. Statistical analysis

All values are expressed as mean±S.E.M. Statistical analysis was performed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The results were considered statistically significant if $P<0.05$.

3. Results

3.1. HPLC analysis of methanol extract of FTB for phenolic profile

The methanol extract of *F. talboti* bark may contains

group of chemical compounds. To establish a better characterization of methanol extract was carried out for HPLC analysis. HPLC study revealed that, FTB methanol extract shows the antidiabetic constituent of Rutin, Quercetin and Kaemfeorl phenolic compound (Figure 1).

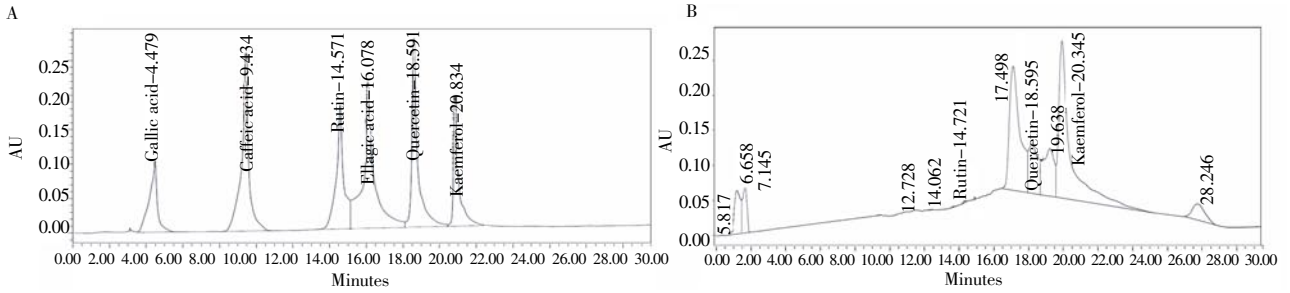

Figure 1. (A) HPLC analysis of gallic acid, caffeic acid, rutin, ellagic acid, quercetin and kaemferol from pure standards detected at a wavelength of 254 nm. (B) HPLC chromatogram of FTB with rutin, quercetin and kaemferol.

3.2. Acute oral toxicity study

In acute toxicity study, FTB treated animals did not show any change in their behavioral pattern. There was no significant difference in the body weight and food consumption when compared to the vehicle treated group. Also, no gross pathological changes were seen. Thus, it was concluded that FTB was safe at 2 000 mg/kg.

3.3. Effect of FTB on normoglycemic rats

Results of the effect of graded doses of FTB on blood glucose level of normal healthy rats are presented in Figure 2. In normoglycemic rats, FTB showed dose dependent hypoglycemic effect at 3 h. Dose dependent blood glucose reduction was observed in animals treated with 100 mg/kg and 200 mg/kg (24.70% and 26.1%, respectively). Blood glucose levels were restored in all treatment groups by 4h. From this study, it could be conclude that 200 mg/kg showed the maximum improvement in glucose tolerance test.

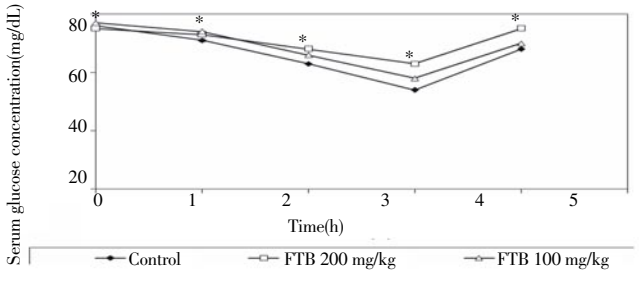

Figure 2. Effect of FTB on blood glucose levels in normoglycemic rats. Each value is expressed as mean of six observations. *P<0.05 when compared with values of 0h of the same group.

3.4. Effect of FTB on oral glucose tolerance in normal rats

FTB, when administered 60 min. prior to glucose loading produced significant reduction (P<0.05) in the rise in blood glucose levels at 60 min. after glucose administration. FTB at doses of 100 and 200 mg/kg produced 13.32% and 19.15% reduction in blood glucose respectively when compared to vehicle treated group at 60 min (Figure 3).

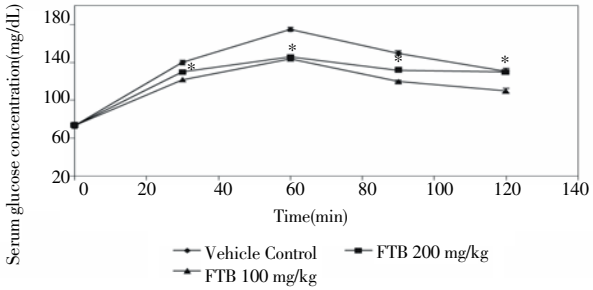

Figure 3. Effect of FTB on oral glucose tolerance in rats. Each value is expressed as mean of six observations. *P<0.05 when compared with corresponding values of the control group.

3.5. Effect of FTB on fasting blood glucose and body weight in STZ-induced diabetic rats

The effect of repeated oral administration of FTB on blood glucose levels in STZ-diabetic rats is presented in Figure 4. FTB, administered at two different doses of 100 mg/kg and 200 mg/kg to STZ-treated diabetic rats caused significant (P< 0.001) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 21 (45.60% and 47.50%, respectively). FTB 200 mg/kg exhibited maximum glucose lowering effect in diabetic rats compared to the other two doses. Glibenclamide exhibited 61.66% reduction in blood glucose levels at the end of the study when compared to diabetic control. STZ produced significant loss in body weight as compared to normal animals during the study. Diabetic control continued to lose weight till the end of the study while FTB at all the two doses (100 mg/kg and 200 mg/kg) showed significant improvement (P<0.05) in body weight compared to diabetic control (Figure 5).

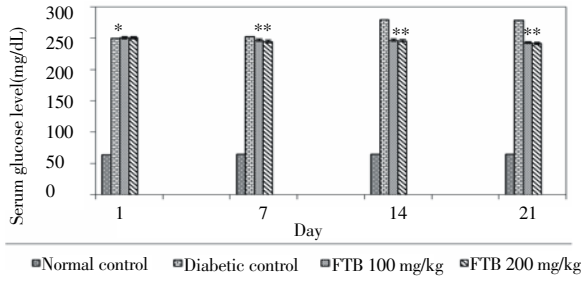

Figure 4. Effect of FTB on blood glucose levels of STZ-induced diabetic rats.

Each value is expressed as mean±S.E.M. (n=6). *P<0.001 when compared to corresponding values of the normal control.

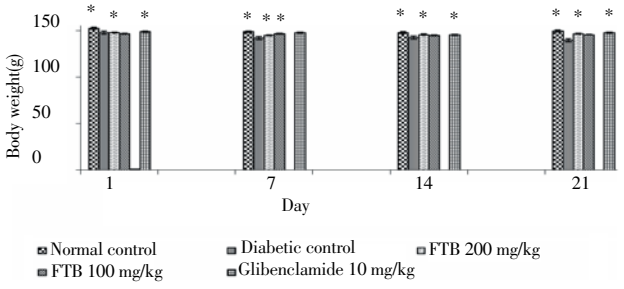

Figure 5. Effect of FTB on body weight of STZ-induced diabetic rats.

Each value is expressed as mean±S.E.M. (n=6). *P<0.05 when compared to corresponding values of the normal control.

3.6. Effect of FTB on serum insulin in STZ-induced diabetic rats

STZ caused a significant decrease in serum insulin. Administration of FTB at all the two doses (100 mg/kg and 200 mg/kg) caused significant (P<0.01) increase in insulin levels at the end of the study. Of the two doses, 200 mg/kg showed maximum increase which was comparable to glibenclamide (Table 1).

3.7. Effect of FTB on serum lipids in STZ-induced diabetic rats

FTB showed a dose related significant (P<0.01) reduction in triglycerides (20.62% and 25.12% for 100 mg/kg and 200 mg/kg, respectively) compared to pretreatment levels (Table 1). FTB at the doses of 200 mg/kg was more effective than 100 mg/kg in reducing the cholesterol levels.

3.8. Effect of FTB on glycogen content in STZ-induced diabetic rats

Glycogen content in liver and skeletal muscle decreased significantly (P<0.001) in diabetic control compared to normal control (Table 1). Administration of FTB at the doses of 100 mg/kg and 200 mg/kg for 21 days resulted in significant (P<0.001) increase in the glycogen levels in both the liver and skeletal muscle. However, with none of the dose levels, the values were restored to normal.

Table 1

Effect of FTB on serum insulin, serum lipids, glycogen content in liver and skeletal muscle and lipid peroxidation in pancreas of STZ treated diabetic rats.

Experimental group	Serum insulin (μU/mL)		Triglyceride (mg/dL)		Total cholesterol (mg/dL)		Glycogen (mg/g of wet tissue)		TBARS (μM/g of wet tissue)
	Day 1	Day 21	Day 1	Day 21	Day 1	Day 21	Liver	Skeletal muscle	
Normal control	19.22±0.20	18.58±0.44	80.24±2.38	84.78±5.02	59.00±1.20	69.00±3.21	46.42±1.32	7.46±0.24	0.37±0.02
Diabetic control	7.52±0.26	6.02±0.30	162.10±5.62	220.11±6.67	125.10±1.32	151.41±5.90	14.30±1.12[d]	4.50±0.12[d]	0.70±0.02[d]
Diabetic+ FTB (100 mg/kg)	8.11±0.40	14.77±0.41[a,c]	163.40±4.15	131.24±5.52[a,c]	99.67±2.84	57.66±2.79[b,c]	27.73±1.12[c]	4.72±0.11[c]	0.45±0.02[c]
Diabetic+ FTB (200 mg/kg)	8.14±0.42	15.80±0.51[a,c]	156.56±8.32	118.42±4.71[a,c]	113.43±1.52	60.40±3.78[b,c]	28.71±1.24[c]	4.59±0.11[c]	0.43±0.01[c]
Diabetic+ glibenclamide (10 mg/kg)	8.18±0.43	19.31±0.30[a,c]	151.01±7.45	101.41±3.41[a,c]	99.19±2.13	52.91±2.80[b,c]	32.60±0.97[c]	5.06±0.10[c]	0.44±0.03[c]

FTB — *Ficus talboti* bark, STZ- Streptozotocin, TBARS-Thiobarbituric acid reactive substances; Each value is mean±S.E.M. (n=6). [a]P<0.01 when compared to the day 1 values of the same group; [b]P < 0.001 when compared to the day 1 values of the same group; [c]P<0.001 when compared to the corresponding values of the diabetic control; [d]P<0.001 when compared with the corresponding values of the normal control.

3.9. Effect of FTB on pancreatic lipid peroxidation in STZ-induced diabetic rats

There was a significant elevation in the level of TBARS in the diabetic control when compared with the corresponding normal control. However, the oral administration of FTB and glibenclamide tended to bring these values back to normal (Table 1).

3.10. Effect of FTB on SGOT, SGPT, ALP, CAT, SOD and GPx

In STZ induced diabetic rats the effect was a significant increase in activities of SGOT, SGPT and ALP was observed in diabetic rats. After treatment with FTB at 100 and 200 mg/kg the SGOT, SGPT and ALP activities were significantly (P <0.001) reduced compared to diabetic

control rats. Table 2 shows the activities of CAT, SOD and GPx and levels of normal and diabetic rats. A significant (P<0.001) reduction in the activities of CAT, SOD and GPx, an increase in the levels were seen in diabetic rats. Oral administration of FTB (100 and 200 mg/kg) brought the values near to normal which is similar in case of Glibenclamide.

3.11. Histopathological Studies

Histopathological studies of liver revealed that FTB significantly enhanced the lobular architecture and central vein. The histological results of pancreas exposed extensively improved architecture of the islets of Langerhans (Figure 6 and Figure 7). The groups treated with FTB showed greater persistence of the islets and lesser degree of necrotic changes as compared to the untreated STZ diabetic rats. STZ causes selective degeneration of pancreatic β-cells in experimental diabetes, in that way inhibiting insulin secretion. In the present study, the hypoglycemic action of FTB depended on insulin secretion.

Table 2

Changes in SGOT, SGPT, ALP, SOD, CAT, GPx and total protein of normal and diabetic rats after 21 days of treatment with *F. talboti* bark.

Experimental group	SGOT (U/dL) #	SGPT (U/dL) #	ALP (U/dL) #	SOD (U/mg protein)	CAT (mM H_2O_2 decomposed/ min)	GPx (U/mg protein)	Total protein (mg/dL)
Normal control	35.42±2.44	54.52±5.40	50.60±2.42	4.28±0.02	61.71±2.93	42.35±1.63	7.72±0.52
Diabetic control	65.42±2.38	90.45±2.42	85.32±3.20	2.92±0.03	35.30±2.93	23.71±1.42	4.94±0.65
Diabetic+ FTB (100 mg/kg)	53.39±4.32	80.03±3.85	69.20±4.38	3.54±0.04[a]	39.35±1.78	33.42±1.55[a]	5.69±0.57
Diabetic+ FTB (200 mg/kg)	48.41±2.41[a]	55.32±2.27[b]	67.20±3.42	4.07±0.03[a]	54.59±1.85[a]	38.30±1.13[a]	6.87±0.62
Diabetic+ glibenclamide (10 mg/kg)	44.30±5.40[a]	55.29±3.38[b]	58.60±2.72[b]	4.40±0.04[a]	57.50±1.94[a]	40.30±1.34[a]	9.10±0.54[b]

SGOT – Serum glutamic oxaloacetic transaminase; SGPT – Serum glutamic pyruvic transaminase; ALP – Alkaline phosphatase; SOD – Superoxide dismutase; CAT – Catalase; GPx – Glutathione peroxidase; # Each value is mean±S.E.M. (n=6); [a]P<0.01 when compared to the day 1 values of the same group; [b]P<0.001 when compared to the day 1 values of the same group.

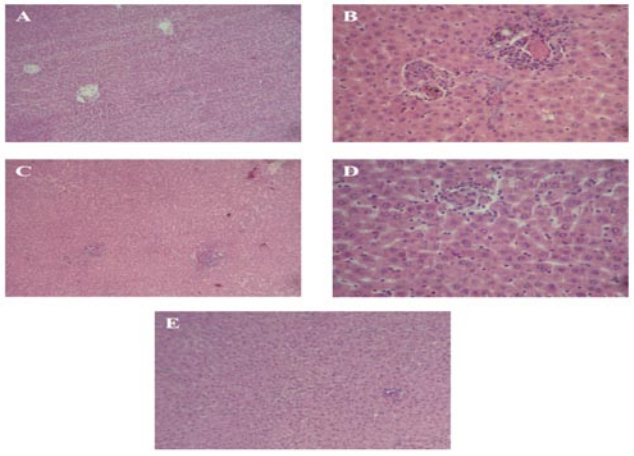

Figure 6. Histology of liver in experimental rats after 21 days of treatment.

(A)Normal control section shows the normal liver showing normal hepatic cells and architecture. (B) Diabetic control section shows the Clear hepatocellular necrosis and extensive vacuolization with vanishing of nuclei and disordered liver structure in STZ induced rat. (C) Diabetic + FTB (100 mg/kg) section liver shows normal hepatocellular architecture with normal nucleus and cytoplasm. (D) Diabetic + FTB (200 mg/kg)–FTB section significantly attenuated STZ–induced hepatocellular necrosis and fibrotic changes in rat liver. (E) Diabetic + glibenclamide (10 mg/kg) – section of liver showing normal hepatocellular architecture with normal nucleus, cytoplasm and distinct hepatic layer.

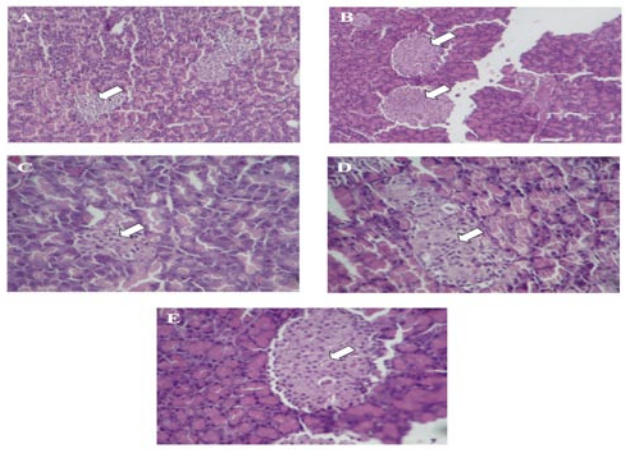

Figure 7. Histology of pancreas in experimental rats after 21 days of treatment.

(A) Normal control section presence of normal pancreatic islet cells. (B) Diabetic control – degranulated and dilated islet cells. (C) Diabetic + FTB (100 mg/kg) section shows the granulated pancreatic islets, showing prominent hyper plasticity islet. (D) Diabetic + FTB (200 mg/kg) section showed the Pancreas showing islets with endocrine cells showing more cytoplasm and normal exocrine acini. (E) Diabetic + glibenclamide (10 mg/kg) section shows the granulated, nonappearance of dilation and important hyper plasticity of islets.

4. Discussion

STZ-induced hyperglycemia has been described as an utilizable experimental model to study the activity of hypoglycemic agents. In our study, a significant decrease in blood glucose and increase in plasma insulin levels were observed in diabetic rats treated with FTB. This could be due to the potentiation, by the extract, of the pancreatic secretion of insulin from regenerated b-cells, or its action to release bound insulin from regenerated b-cells by inhibiting ATP sensitive K^+ channels like glibenclamide. STMe contained copious amount of phenolic compounds. Previous studies showed that phenolic compounds acted on ATP sensitive K^+ channels and regulated blood glucose[23].

This study was undertaken to evaluate the hypoglycemic activity of FTB in normal, glucose-loaded hyperglycemic and streptozotocin-induced diabetic rats. In normoglycemic rats, FTB showed dose dependent hypoglycemic effect at 3 h. From oral glucose tolerance test, it could be concluded that dose of 200 mg/kg showed the maximum improvement in glucose tolerance. STZ significantly induced hyperglycemia accompanied by hypoinsulinemia. Oral administration of FTB for 21 days caused a significant decrease in blood glucose levels. The possible mechanism by which FTB mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. The hypoglycemic activity of FTB was compared with glibenclamide, a standard hypoglycemic drug. From the results of the present study, it may be suggested that the mechanism of action of FTB may be similar to glibenclamide action.

Diabetes mellitus impairs the normal capacity of the liver to synthesize glycogen. Synthase phosphatase activates glycogen synthase resulting in glycogenesis and this activation appears to be defective in diabetes[24]. Skeletal muscle is also a major site of insulin-stimulated glucose uptake[25]. Decrease in both muscle and hepatic glycogen was observed in this study. Treatment with FTB (100 mg/kg and 200 mg/kg) for 21 days significantly increased muscle and liver glycogen indicating that the defective glycogen storage of the diabetic state was partially corrected by the extract.

Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes[26]. FTB significantly reduced serum triglycerides and total cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that FTB could modulate blood lipid abnormalities.

In diabetes, tissue damage is considered to be mediated by free radicals by attacking membranes through peroxidation of unsaturated fatty acids[27]. Lipid peroxidation eventually leads to extensive membrane damage and dysfunction[28]. Decreased lipid peroxidation and improved antioxidant status may be one of the mechanisms by which drug treatment could contribute to the prevention of diabetic complications[29]. In our study, FTB significantly attenuated the increased lipid peroxidation which could be due to the antioxidant effect of flavonoids, detected in the preliminary phytochemical screening of the extract.

Diabetic animals showed decreased activity of the key antioxidant enzymes viz. SOD, CAT and GPx, which play an important role in scavenging the toxic intermediates of incomplete oxidation. STZ treatment causes significant increases in lipid peoxidation (MDA) and nitric oxide (NO) generation, and decreases antioxiadant enzyms such as catalase, Glutatahione peroxidase, and superoxide dismutase activities as well as pancreatic insulin contents when compared with the control animals in experiments. Decreases in antioxidant activities, and simultaneous increases in MDA and NO activities, reflect susceptibility of pancreas to STZ's significant oxidative stress[30]. FTB treated diabetic rats showed the enhanced antioxidant enzyme levels and pancreatic insulin contents.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotics and their metabolites. SGOT, SGPT and ALP are reliable markers of liver function. In STZ-induced diabetic rats the liver was necrotized. An increase in the activities of SGOT, SGPT and ALP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream which gives an indication of the hepatotoxic effect of STZ[31]. Treatment of the diabetic rats with FTB reduced the activity of these enzymes in plasma compared to the diabetic untreated group and consequently alleviated liver damage caused by STZ-induced diabetes. Significant reductions in the activities of these enzymes in FTB treated diabetic rats indicated the hepatoprotective role in preventing diabetic complications.

An increase in the body weight of diabetic treated rats might be due to an enhancement in glycemic control and increased synthesis of structural proteins[32]. Reduction in total protein content was observed in diabetic rats which might be due to progressive protenuria[33]. FTB treatment considerably normalized the total protein in diabetic rats suggesting its curative role on kidney function. The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in

muscle wasting and weight loss in diabetic untreated rats. In streptozotocin-induced diabetic rats, improved food consumption and decreased body weight were observed. This indicates polyphagic condition and loss of weight due to excessive break-down of tissue proteins[34]. Hakim *et al.* [35] have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats[36]. Rutin administration to diabetic rats decreased food consumption and improved body weight and this could be due to a better control of the hyperglycaemic state in the diabetic rats. Decreased levels of blood glucose could improve body weight in streptozotocin-diabetic rats [37]. Streptozotocin administration to rats increased blood glucose and decreased insulin and C-peptide levels. Rutin treated streptozotocin-diabetic rats exhibited a decrease in plasma glucose and an increase in insulin and C-peptide levels. Rutin by its ability to scavenge free radicals and to inhibit lipid peroxidation, prevents streptozotocin-induced oxidative stress and protects β-cells resulting in increased insulin secretion and decreased blood glucose levels.

Kaempferol and some glycosides of kaempferol may also decrease triglycerides levels, cholesterol levels and reduce body weight[38]. Evidence suggests that some kaempferol glycosides and several kaempferol-containing plants have antidiabetic activity[39] and may prevent diabetic complications[40]. The HPLC analysis report of FTB showed the presence of Rutin, Quercetin and Kaemfeorl. Thus, the significant antidiabetic effect of FTB may be due to the presence of Rutin, Quercetin and Kaemfeorl, which alone or in synergism can impart therapeutic effect. In the present study, the hypoglycemic action of FTB depended on insulin secretion. This could be due to the insulin secretagogue effect of antihyperglycemic constituents, kaempferol. The results of this study also demonstrated that FTB had regenerative potential with increased insulinimmunoreactive β-cells in STZ-induced diabetic rats.

In the present study, the hypoglycemic action of FTB has beneficial effects on blood glucose level. It also restored the altered serum enzymes (SGOT, SGPT and ALP), total protein levels to near normal. FTB significantly enhanced the levels of endogenous antioxidant enzymes (GPx, CAT and SOD). The action of FTB was comparable to the antidiabetic drug glibenclamide. The HPLC analysis showed the biologically active compound of Rutin, Quercetin and Kaemfeorl. This may be the reason for FTB has an effective antioxidant and antidiabetic activity. Overall results of this experimental study indicated that FTB possessed good antioxidant and antidiabetic activities. This study results may possibly used for the development of pharmaceutical drug for type I diabetic disease. Further pharmacological and biochemical investigations are under way to elucidate the mechanism of the antidiabetic effect of FTB.

Conflict of interest statements

We declare that we have no conflict of interest.

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