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Antihyperglycemic and Antilipidperoxidative Effects of *Ficus racemosa* (Linn.) Bark Extracts in Alloxan Induced Diabetic Rats

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Different parts of the *Ficus racemosa* plant products are employed in the Indian traditional medicine for the treatment of several disorders including diabetes mellitus. Our aim was to investigate the antihyperglycemic and antilipidperoxidative effects of aqueous and ethanolic extracts of *Ficus racemosa* bark (FrABet and FrEBet) in alloxan induced diabetic rats. Oral administration of FrABet and FrEBet at a dose of 400 and 300 mg kg^{-1} bw, respectively showed potent antihyperglycemic and antilipidperoxidative effects in alloxan induced diabetic rats. The extracts also improved the antioxidants defense system in alloxan induced diabetic rats. The FrEBet showed better effect than glibenclamide whereas the effect of FrABet was much comparable to that of glibenclamide (reference drug). Our results indicate that FrABet and FrEBet have prominent antidiabetic and antilipidperoxidative effects in experimental diabetes and can therefore be used as an alternative remedy for the treatment of diabetes mellitus and its complications.

Key words: Diabetes mellitus, lipidperoxidation, antioxidants, *Ficus racemosa*, alloxan

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INTRODUCTION

Diabetes mellitus, a group of metabolic disorder with multiple aetiology, is characterized by chronic hyperglycemia with disturbances in carbohydrate, fat and protein metabolism, due to defects in insulin secretion, insulin action or both. Diabetes is mainly associated with low blood insulin level or insensitivity of target organs to insulin (Alberti and Zimmet, 1998). World Health Organization (WHO) has reported that the diabetic population will increase to 300 million or more by the year 2025 (Boyle *et al*., 2001). As the prevalence of type I diabetes is low in Asian, Indian, middle eastern and African population, type II diabetes would constitute well over 90% of diabetic cases (Cockram, 2000). It has also been reported that India and China will be the leading countries in their annual incidence rates for diabetes mellitus by the year 2025 due to their immense population (King *et al*., 1998). Alloxan, a simple nitrogenous organic compound, can produce diabetes mellitus in laboratory animals by single intraperitoneal injection. Although the precise diabetogenic mechanism of alloxan has not yet been fully understood, several studies indicated that pancreatic β-cell oxidative damage mediated by alloxan could play a possible role (McLetchie, 2002).

In recent years, free radical induced lipid peroxidation has become an area of interest in understanding the process of human diseases. Reactive oxygen species interfere with the structure and function of the cells making them weak and defenseless. A mismatch between the production of prooxidants and antioxidants in cells could lead to serious cellular damage and has been implicated in the pathogenesis of several disorders including diabetes mellitus (Giugliano *et al*., 1996; Mohan and Das, 1997). The deleterious effects of Reactive Oxygen Species (ROS) induced oxidative damage are neutralized by antioxidant systems, which are classified as enzymatic [Superoxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx)] and nonenzymatic [Vitamin E, Vitamin C and Reduced glutathione (GSH)] defense systems (Fang *et al*., 2002). Over production of lipid peroxides and decline in antioxidants have been well documented in both type I and type II diabetes mellitus (Ruiz *et al*., 1999; Seghrouchni *et al*., 2002).

The use of herbal medicine is widespread and traditionally several medicinal plants are being used to treat diabetic patients. The world health organization has estimated that the majority of the world's population depends on herbal medicines for basic health care needs. Phytochemicals, identified from traditional medicinal plants, are presenting an exciting opportunity for the development of new types of therapeutics. Although a large number of medicinal plants have been already tested for their antidiabetic and antilipidperoxidative effects, these effects remain to be investigated in several other Indian medicinal plants.

Ficus racemosa, an evergreen 15-18 m-height tree, grows along the bank of rivers and streets. It is popularly known as Country fig in English and Atti in Tamil. Different parts of *F. racemosa* [bark, fruits and root] are used in folk medicine for the treatment of several diseases including diabetes mellitus (Joshi, 2000). Experimental studies have demonstrated the anti-inflammatory, hepatoprotective and hypoglycemic effects of *F. racemosa* (Li *et al*., 2004; Mandal *et al*., 1999; Bhaskara Rao *et al*., 2002). The present study was therefore designed to evaluate the antihyperglycemic effect of ethanolic and aqueous extracts of *F. racemosa* bark in alloxan induced diabetic rats. The mechanistic pathway for their antidiabetic role was also assessed by evaluating the status of carbohydrate metabolizing enzymes, lipidperoxidation byproducts and antioxidants in blood and tissues as biochemical end points.

MATERIALS AND METHODS

Animals: Albino wistar male rats 7 to 8 weeks old and weighing 150-200 g were used for the present study. The animals were obtained from Central Animal House, Rajah Muthiah Institute of Health Sciences, Annamalai University, India and were maintained at 12 h light-dark cycles. The animals were randomized into control and experimental groups and were housed 4 or 5 in a polypropylene cage. Standard pellets obtained from Mysore Snack Feed Ltd., Mysore, India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water *ad libitum*.

Chemicals: Alloxan was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Plant material: *Ficus racemosa* bark was collected in and around Chidambaram, Tamil Nadu and identified by the Botanist, Dr. S. Sivakumar, Reader, Department of Botany, Annamalai University. A voucher specimen was deposited in the Department of Botany, Annamalai University.

Preparation of the plant extracts

Aqueous extract preparation [FrABet]: One hundred grams of *F. racemosa* bark was air-dried, powdered and

suspended in 250 mL of water for 2 h and then heated at 60-65°C for 30 min. The extract was collected and the process was repeated three times with the residual powder, each time collecting the extract. The collected extract was pooled and passed through fine cotton cloth. The filtrate upon evaporation at 40°C yielded 11% semisolid extract, which was stored at 0-4°C until used.

Ethanolic extract preparation [FrEBet]: Five hundred grams of fresh bark of *F. racemosa* was dried, powdered and then soaked in 1500 mL of 95% of ethanol overnight. After filtration, the residue obtained was again resuspended in equal volume of 95% ethanol for 48 h and filtered again. The above two filtrates were mixed and the solvent was evaporated in a rotavapor at 40-50°C under reduced pressure. A 7% semisolid dark brown material obtained was stored at 0-4°C until used.

A known volume of FrABet and FrEBet is suspended in distilled water and was orally administered to the animals by gastric intubation using a force-feeding needle during the experimental period.

Induction of diabetes mellitus: Diabetes mellitus was induced in overnight fasted Wistar rats by single intraperitoneal injection of freshly prepared solution of alloxan monohydrate (150 mg kg^{-1} bw) in physiological saline (Al -Shamaony *et al*., 1994).

Experimental design: The local institutional animal ethics committee, Annamalai University, Annamalainagar, India approved the experimental design.

A total number of 42 rats (24 diabetic rats, 18 control rats) were used and the rats were divided into 7 groups of six each.

Group I served as control animals and received 2 mL of distilled water (instead of FrABet and FrEBet) by gastric intubation using force-feeding needle.

Group II animals were treated with single intraperitoneal injection of a lloxan monohydrate (150 mg kg^{-1} bw) after overnight fast for 12 h. Determining the blood glucose concentration 3 days and 5 days after alloxan treatment assessed the diabetic condition. The rats with blood glucose level above 260 mg dL^{-1} and urinary sugar (+++) were selected for the experimental study.

Group III animals were received 2 mL water solution of FrABet (400 mg kg^{-1} bw in 2 mL distilled water) once daily for 45 days after the diabetic state was assessed in alloxan induced diabetic rats.

Group IV animals were received 2 mL water solution of FrEBet (300 mg kg^{-1} bw in 2 mL distilled water) once daily for 45 days after the diabetic state was assessed in alloxan induced diabetic rats.

Group V animals were received the reference drug, glibenclamide (600 μg kg^{-1} bw) in 2 mL of distilled water once daily for 45 days after diabetic state was assessed in alloxan induced diabetic rats.

Group VI animals were treated with FrABet (400 mg kg^{-1} bw) alone in 2 mL-distilled water for 45 days in order to evaluate the hypoglycemic effect of the FrABet in control rats.

Group VII animal were treated with FrEBet (300 mg kg^{-1} bw) alone in 2 mL-distilled water for 45 days in order to evaluate the hypoglycemic effect of the FrEBet in control rats.

After the experimental period, all animals were sacrificed by cervical dislocation and biochemical studies were conducted in blood, plasma, erythrocytes, erythrocyte membrane, liver and kidney of control and experimental animals in each group.

Biochemical estimations: Plasma was separated from collected blood samples by centrifugation at 3000 rpm for 15 min. After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocytes was lysed with hypotonic phosphate buffer at pH 7.4. The hemolysate was separated by centrifugation at 10,000 rpm for 15 min at 20°C. The erythrocyte membrane was isolated according to the procedure of Dodge *et al*. (1968) with a change in buffer according to Quist (1980). Liver and kidney samples from animals were weighed and homogenized using appropriate buffer in an all glass homogenizer with Teflon pestle using specified medium and then used for biochemical estimations.

Blood glucose was determined by the method of Sasaki *et al*. (1972) using O-toluidine reagent. Total haemoglobin and glycosylated haemoglobin were determined by the methods of Drabkin and Austin (1932) and Sudhakar Nayak and Pattabiraman (1981), respectively. Plasma insulin was determined by ELISA method using Boehinger Mannheim GmbH Kit (Anderson *et al*., 1993). Liver glycogen content was measured by the method of Morales *et al*. (1973). The activity of hexokinase (Brandstrup *et al*., 1957), glucose-6-phosphatase (Koida and Oda, 1959), glucose-6-phosphate dehydrogenase (Ellis and Kirkman, 1961), Fructose-1, 6-bis phosphatase (Gancedo and Gancedo, 1971) and glycogen phosphorylase (Shull *et al*., 1956) were estimated according to the methods, respectively.

TBARS in plasma, erythrocyte membranes and tissues (liver and kidney) were assayed according to the methods of Yagi (1978), Donnan (1950) and Ohkawa *et al*. (1979), respectively. Lipid hydroperoxides in plasma, liver and kidney were assayed according to the method of Jiang *et al*. (1992). The levels of non-enzymatic

antioxidants, Vitamin C and reduced glutathione were measured according to the methods of Omaye *et al.* (1979) and Beutler and Kelley (1963), respectively. The activities of enzymatic antioxidants, superoxide dismutase, catalase and glutathione perioxidase were determined according to the methods of Kakkar *et al.* (1984), Sinha (1972) and Rotruck *et al.* (1984), respectively.

Statistical analysis: The data are expressed as mean±SD. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p values were less than 0.05.

RESULTS

Table 1 shows the status of blood glucose, urinary sugar, total haemoglobin, glycosylated haemoglobin, plasma insulin and liver glycogen content in control and experimental animals in each group. Blood glucose and glycosylated haemoglobin levels were significantly increased whereas plasma insulin, total haemoglobin and liver glycogen were decreased in diabetic animals as compared to control animals. The urinary sugar was found to be (+++) in diabetic animals. The status of blood glucose, urinary sugar, glycosylated haemoglobin, total haemoglobin, plasma insulin and liver glycogen was restored in diabetic rats after treated with FrABet, FrEBet and glibenclamide. The FrEBet showed better effect than glibenclamide, whereas the effect of FrABet was much comparable to that of glibenclamide. Control rats treated with FrABet and FrEBet alone showed no significant difference in blood picture and liver glycogen content as compared to control rats.

Table 2 shows the activities of glucose-6-phosphatase, hexokinase, glucose-6-phosphate dehydrogenase, fructose-1, 6-bisphosphatase and glycogen phosphorylase in liver and kidney of control and experimental animals in each group. Glucose-6-phosphatase, glycogen phosphorylase and fructose-1,6-bisphosphatase activities were significantly increased whereas the activities of hexokinase and glucose-6-phosphate dehydrogenase were decreased in diabetic rats as compared to control rats. However, the activities of glucose-6-phosphatase, hexokinase, fructose-1,6-bisphosphatase, glucose-6-phosphate dehydrogenase and glycogen phosphorylase were returned to near normal range in diabetic rats treated with FrABet and FrEBet and diabetic rats treated with glibenclamide. The FrEBet showed better effect than glibenclamide whereas the effect of FrABet was much comparable to that of glibenclamide. Control rats treated with FrABet and FrEB et al one showed no significant difference in the activities of enzymes as compared to control rats.

Table 3 shows the levels of TBARS and lipidhydroperoxides (LHP) in plasma, erythrocytes, liver and kidney of control and experimental animals in each group. TBARS and LHP levels were significantly increased in alloxan induced diabetic rats as compared to control rats. Treatment of alloxan induced diabetic rats with FrABet and FrEBet for 45 days normalized the levels of plasma and tissue TBARS and LHP. The FrEBet showed better effect than glibenclamide whereas the effect of FrABet was much comparable to that of glibenclamide. Control rats treated with FrABet and FrEB et al one showed no significant difference in TBARS and LHP levels as compared to control rats.

Table 4 shows the activities of enzymatic antioxidants (Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx) in plasma, erythrocytes, liver and kidney of control and experimental animals in each group. CAT, SOD and GPx activities were significantly decreased in alloxan induced diabetic rats as compared to

Table 1: Blood glucose, urine sugar, plasma insulin, total hemoglobin, glycosylated hemoglobin and liver glycogen concentrations in control and experimental animals in each group

Parameters	Group I control	Group II diabetic control	Group III Diabetic+FrABet (400 mg kg^{-1} bw)	Group IV Diabetic+FrEBet (300 mg kg^{-1} bw)	Group V Diabetic+ Glibenclamide (600 μg kg^{-1} bw)	Group VI Control+FrABet (400 mg kg^{-1} bw)	Group VII Control+FrEBet (300 mg kg^{-1} bw)
Fasting blood glucose (mg dL^{-1})	88.17±7.65^{a}	294.88±28.82^{b}	123.60±11.47^{cd}	106.69±9.14^{c}	122.07±10.21^{c}	90.88±7.90^{a}	85.52±6.75^{a}
Urine sugar*	Nil	+++	Nil	Nil	Nil		Nil
Plasma Insulin (μU mL^{-1})	13.64±1.04^{a}	8.30±0.72^{b}	12.56±0.97^{cd}	12.67±1.27^{ac}	11.44±1.04^{c}	14.58±1.13^{a}	13.70±1.02^{a}
Total hemoglobin (g dL^{-1})	12.77±0.67^{a}	8.16±0.68^{b}	11.33±0.95^{cd}	12.27±0.90^{c}	10.91±0.75^{d}	13.02±1.13^{a}	13.09±0.96^{a}
Glycosylated hemoglobin (HbA1%)	3.60±0.28^{a}	6.83±0.49^{b}	4.02±0.24^{cd}	3.70±0.26^{ac}	4.23±0.33^{d}	3.57±0.19^{a}	3.52±0.20^{a}
Liver glycogen (mg glucose/g tissues)	38.77±3.09^{a}	19.52±1.55^{b}	30.83±2.40^{d}	34.37±2.67^{c}	26.63±1.91^{e}	39.44±2.84^{a}	39.79±3.10^{a}

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT); FrABet-*Ficus racemosa* aqueous bark extract; FrEBet-*Ficus racemosa* ethanolic bark extract; *(+++) Indicates more than 1% Sugar

Table 2: Activities of hexokinase, glucose-6-phosphatase, glucose-6-phosphate dehydrogenase, fructose 1,6 bisphosphatase and glycogen phosphorylase in liver and kidney of control and experimental animals in each group

Parameters	Group I control	Group II diabetic control	Group III Diabetic+FrABet (400 mg kg^{-1} bw)	Group IV Diabetic+FrEBet (300 mg kg^{-1} bw)	Group V Diabetic+ glibenclamide (600 µg kg^{-1} bw)	Group VI Control+FrABet (400 mg kg^{-1} bw)	Group VII Control+FrEBet (300mg kg^{-1} bw)
Liver							
Hexokinase (U^A g^{-1} protein)	141.27±9.99^a	100.49±8.15^b	127.85±10.18^{cd}	135.27±9.68^{ac}	122.55±10.91^d	143.65±11.01^a	147.33±11.83^a
Glucose-6-Phosphtase (U^B mg^{-1} protein)	3.95±0.28^a	5.75±0.38^b	4.46±0.41^{cd}	4.15±0.2^c	4.73±0.39^d	3.79±0.27^a	3.74±0.28^a
Glucose-6-phosphate Dehydrogenase (x10^{-4} mLU mg^{-1} protein)	4.91±0.34^a	2.06±0.17^b	4.22±0.33^{cd}	4.35±0.29^c	3.95±0.27^d	5.13±0.37^a	5.20±0.38^a
Fructose 1,6 bisphosphatase (U^C mg^{-1} protein)	0.32±0.03^a	0.53±0.04^b	0.39±0.03^c	0.36±0.02^c	0.45±0.04^d	0.31±0.03^a	0.30±0.03^a
Glycogen phosphorylase (U^D mg^{-1} protein)	3.55±0.23^a	4.41±0.36^b	3.96±0.31^{cd}	3.69±0.45^{ac}	4.06±0.28^d	3.52±0.26^a	3.50±0.28^a
Kidney							
Hexokinase (U^A g^{-1} protein)	135.85±9.39^a	82.51±6.43^b	115.98±9.37^d	127.02±10.92^c	110.05±9.78^d	136.57±9.00^a	138.58±10.08^a
Glucose-6-phosphate (U^B mg^{-1} protein)	3.29±0.22^a	6.61±0.45^b	3.59±0.32^{cd}	3.45±0.29^{ac}	3.75±0.30^d	3.22±0.25^a	3.20±0.24^a
Glucose-6-phosphate Dehydrogenase (x10^{-4} mLU mg^{-1} protein)	3.95±0.23^a	1.98±0.14^b	3.21±0.27^d	3.59±0.33^c	2.87±0.27^e	4.08±0.29^a	4.07±0.31^a
Fructose 1,6 bisphosphatase (U^C mg^{-1} protein)	0.42±0.03^a	0.65±0.04^b	0.55±0.03^d	0.49±0.02^c	0.58±0.05^e	0.40±0.02^a	0.39±0.02^a
Glycogen phosphorylase (U^D mg^{-1} protein)	3.19±0.26^a	4.09±0.38^b	3.60±0.23^{cd}	3.45±0.26^c	3.75±0.21^d	3.14±0.19^a	3.09±0.21^a

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter(s) differ significantly at p<0.05 (DMRT); A-Micromoles of glucose phosphorylated min^{-1}; B-Micromoles of Pi liberated min^{-1}; C-Micromoles of Pi liberated h^{-1}; D-Micrograms of Pi liberated min^{-1}; FrABet-*Ficus racemosa* aqueous bark extract; FrEBet-*Ficus racemosa* ethanolic bark extract

Table 3: Levels of TBARS and lipid hydroperoxides in plasma, erythrocyte membrane and tissues of control and experimental animals in each group

Parameters	Group I control	Group II diabetic control	Group III Diabetic+FrABet (400 mg kg^{-1} bw)	Group IV Diabetic+FrEBet (300 mg kg^{-1} bw)	Group V Diabetic+ glibenclamide (600 µg kg^{-1} bw)	Group VI Control+FrABet (400 mg kg^{-1} bw)	Group VII Control+FrEBet (300mg kg^{-1} bw)
TBARS							
Plasma (n moL mL^{-1})	1.66±0.11^a	3.15±0.20^b	1.85±0.15^{cd}	1.77±0.09^{ac}	1.96±0.12^d	1.62±0.11^a	1.59±0.09^a
Erythrocyte membrane (n moL mg^{-1} protein)	0.36±0.02^a	1.37±0.11^b	0.51±0.03^{cd}	0.46±0.02^c	0.55±0.03^d	0.33±0.02^a	0.32±0.02^a
Liver (m mol/100 g tissues)	0.69±0.04^a	1.75±0.09^b	0.79±0.05^{cd}	0.73±0.04^{ac}	0.83±0.06^d	0.67±0.04^a	0.65±0.03^a
Kidney (m mol/100 g tissue)	1.25±0.08^a	3.17±0.26^b	1.52±0.09^c	1.42±0.11^c	1.68±0.13^d	1.20±0.09^a	1.17±0.08^a
LHP							
Plasma (x10^{-5} mM dL^{-1})	7.30±0.59^a	15.49±0.13^b	9.14±0.69^c	7.95±0.50^a	12.80±0.92^d	7.28±0.52^a	7.15±0.42^a
Liver (m mol/100 g tissues)	80.25±5.31^a	110.69±9.94^b	93.47±7.28^{cd}	87.74±6.83^{ac}	98.41±8.17^d	79.36±6.48^a	77.32±5.69^a
Kidney (m mol/100 g tissues)	51.63±4.12^a	79.83±4.28^b	62.68±5.89^{cd}	58.63±4.56^c	68.07±4.90^d	51.29±3.69^a	50.86±3.66^a

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter(s) differ significantly at p<0.05 (DMRT); FrABet-*Ficus racemosa* aqueous bark extract; FrEBet-*Ficus racemosa* ethanolic bark extract

control rats. Oral administration of FrABet and FrEBet for 45 days to diabetic rats restored the activities of CAT, SOD and GPx in plasma, erythrocytes, liver and kidney. The FrEBet showed better effect than glibenclamide whereas the effect of FrABet was much comparable to that of glibenclamide. Wistar rats treated with FrAB et and FrEB et al one showed no significant difference in enzymatic antioxidants activities as compared to control rats.

Table 5 shows the levels of nonenzymatic antioxidants (vitamin-C and glutathione) in plasma, erythrocytes, liver and kidney of control and experimental animals in each group. The vitamin-C and glutathione levels were significantly decreased in plasma, liver and kidney of diabetic rats as compared to control animals. The levels of vitamin- C and glutathione were returned to near normal range in diabetic rats treated with FrABet and FrEBet and diabetic rats treated with glibenclamide. The

Table 4: Activities of enzymatic antioxidants in plasma, erythrocytes, liver and kidney of control and experimental animals in each group

Parameters	Group I control	Group II diabetic control	Group III Diabetic+FrABet (400 mg kg^{-1} bw)	Group IV Diabetic+FrEBet (300 mg kg^{-1} bw)	Group V Diabetic+ glibenclamide (600 μg kg^{-1} bw)	Group VI Control+FrABet (400 mg kg^{-1} bw)	Group VII Control+FrEBet (300mg kg^{-1} bw)
SOD							
Plasma (U^{A} mL^{-1})	5.11±0.54^{a}	3.14±0.25^{b}	4.54±0.41^{c}	4.87±0.47^{ac}	4.42±0.38^{c}	5.13±0.47^{a}	5.16±0.46^{a}
Erythrocytes (U^{A} mg^{-1} Hb)	4.03±0.45^{a}	1.13±0.09^{b}	3.52±0.31^{cd}	3.81±0.37^{ac}	3.31±0.29^{d}	4.07±0.36^{a}	4.13±0.36^{a}
Liver (U^{A} mg^{-1} protein)	5.74±0.52^{a}	3.19±0.25^{b}	5.07±0.42^{c}	5.62±0.55^{a}	4.68±0.40^{c}	5.77±0.44^{a}	5.81±0.46^{a}
Kidney (U^{A} mg^{-1} protein)	14.32±1.20^{a}	8.61±0.66^{b}	13.00±0.92^{c}	13.6±0.97^{ac}	12.82±0.91^{c}	14.34±1.11^{a}	14.43±1.16^{a}
CAT							
Plasma (U^{B} mL^{-1})	0.76±0.06^{a}	0.33±0.03^{b}	0.68±0.05^{c}	0.73±0.05^{ac}	0.59±0.06^{d}	0.77±0.04^{a}	0.78±0.06^{a}
Erythrocytes (U^{B} mg^{-1} Hb)	1.68±0.11^{a}	0.96±0.07^{b}	1.54±0.09^{cd}	1.63±0.14^{ac}	1.48±0.08^{d}	1.69±0.07^{a}	1.71±0.07^{a}
Liver (U^{B} mg^{-1} protein)	71.16±5.07^{a}	37.02±3.80^{b}	64.95±4.18^{cd}	68.29±5.39^{ac}	59.77±5.33^{d}	72.01±6.71^{a}	72.82±5.22^{a}
Kidney (U^{B} mg^{-1} protein)	33.74±3.52^{a}	16.55±1.32^{b}	27.12±2.29^{cd}	29.92±2.29^{c}	24.62±2.33^{d}	34.93±3.02^{a}	35.54±3.18^{a}
GPx							
Plasma (U^{C} L^{-1})	141.93±12.63^{a}	96.10±8.15^{b}	123.60±10.10^{cd}	131.18±9.39^{ac}	117.58±10.02^{d}	142.27±10.82^{a}	143.40±11.08^{a}
Erythrocytes (U^{C} mg^{-1} Hb)	13.07±0.95^{a}	7.34±0.56^{b}	11.91±0.78^{cd}	12.80±0.91^{ac}	11.55±1.0^{d}	13.09±0.95^{a}	13.14±1.01^{a}
Liver (U^{C} L^{-1} protein)	5.35±0.42^{a}	3.27±0.21^{b}	4.78±0.37^{cd}	5.05±0.40^{ac}	4.58±0.33^{d}	5.41±0.46^{a}	5.43±0.37^{a}
Kidney (U^{C} L^{-1} protein)	3.78±0.26^{a}	2.03±0.18^{b}	3.14±0.19^{d}	3.49±0.22^{c}	2.95±0.24^{d}	3.81±0.20^{a}	3.87±0.21^{a}

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT); A-The amount of enzyme required to inhibit 50% NBT reduction; B-Micromoles of H_2O_2 utilized/Sec; C-Micromoles of glutathione utilized min^{-1}; FrABet-*Ficus racemosa* aqueous bark extract; FrEBet-*Ficus racemosa* ethanolic bark extract

Table 5: Levels of non enzymatic antioxidants in plasma, erythrocyte membrane, liver and kidney of control and experimental animals in each group

Parameters	Group I control	Group II diabetic control	Group III Diabetic+FrABet (400 mg kg^{-1} bw)	Group IV Diabetic+FrEBet (300 mg kg^{-1} bw)	Group V Diabetic+ glibenclamide (600 μg kg^{-1} bw)	Group VI Control+FrABet (400 mg kg^{-1} bw)	Group VII Control+FrEBet (300mg kg^{-1} bw)
Vitamin C							
Plasma (mg dL^{-1})	1.75±0.14^{a}	0.71±0.07^{b}	1.54±0.11^{cd}	1.65±0.14^{ac}	1.46±0.12^{d}	1.77±0.13^{a}	1.78±0.15^{a}
Liver (mg/100 mg tissues)	1.47±0.12^{a}	0.72±0.07^{b}	1.23±0.08^{cd}	1.32±0.09^{c}	1.19±0.09^{d}	1.50±0.13^{a}	1.54±0.11^{a}
Kidney (mg/100 mg tissues)	1.20±0.11^{a}	0.44±0.06^{b}	0.81±0.05^{cd}	0.96±0.09^{c}	0.75±0.04^{d}	1.26±0.17^{a}	1.25±0.09^{a}
Vitamin E							
Plasma (mg dL^{-1})	1.48±0.12^{a}	2.68±0.21^{b}	1.65±0.16^{c}	1.56±0.09^{ac}	1.66±0.08^{c}	1.44±0.12^{a}	1.43±0.09^{a}
Erythrocyte membrane (μg mg^{-1} protein)	2.43±0.17^{a}	1.32±0.12^{b}	2.23±0.20^{cd}	2.35±0.19^{ac}	2.10±0.16^{d}	2.44±0.18^{a}	2.45±0.22^{a}
Liver (mg/100 mg tissues)	0.63±0.05^{a}	1.16±0.08^{b}	0.71±0.06^{cd}	0.66±0.05^{ac}	0.74±0.07^{d}	0.61±0.05^{a}	0.60±0.05^{a}
Kidney (mg/100 mg tissues)	0.42±0.03^{a}	0.81±0.07^{b}	0.55±0.04^{c}	0.51±0.04^{c}	0.62±0.05^{d}	0.41±0.03^{a}	0.39±0.03^{a}
GSH							
Plasma (mg dL^{-1})	28.30±2.26^{a}	12.41±1.06^{b}	21.59±1.88^{c}	26.85±2.21^{a}	18.10±1.30^{d}	28.49±2.62^{a}	28.67±1.96^{a}
Erythrocyte membrane (mg dL^{-1})	44.69±3.54^{a}	24.57±2.11^{b}	36.46±2.56^{cd}	39.97±3.30^{c}	33.10±2.37^{d}	45.62±3.52^{a}	47.37±3.24^{a}
Liver (mg/100 mg tissues)	44.54±3.42^{a}	17.52±1.49^{b}	32.63±2.95^{cd}	37.63±2.60^{c}	30.23±2.17^{d}	46.55±2.93^{a}	47.48±3.14^{a}
Kidney (mg/100 mg tissues)	39.67±3.30^{a}	18.66±1.60^{b}	31.02±2.02^{cd}	33.89±2.91^{c}	28.45±2.37^{d}	39.79±3.10^{a}	40.13±2.74^{a}

Values are given as mean±SD (n = 6 rats); Values not sharing a common superscript letter(s) differ significantly at $p<0.05$ (DMRT); FrABet-*Ficus racemosa* aqueous bark extract; FrEBet-*Ficus racemosa* ethanolic bark extract

FrEBet showed better effect than glibenclamide whereas the effect of FrABet was much comparable to that of glibenclamide. Control rats treated with FrABet and FrEBet al one showed no significant difference in vitamin-C and glutathione levels as compared to control rats.

DISCUSSION

In the present study, oral administration of FrABet and FrEBet brought back the levels of blood glucose, plasma insulin, glucosylated and total haemoglobin, carbohydrate metabolizing enzyme patterns and lipid peroxidation and antioxidant status to near normal range in alloxan induced diabetic rats. Increased levels of blood glucose and decreased plasma insulin in diabetic rats are either due to decreased utilization or defect in the activities of carbohydrate metabolizing enzymes (glucose-6-phosphatase, hexokinase, fructose-1, 6-bisphosphatase, glucose-6-phosphate dehydrogenase and glycogen phosphorylase). The antihyperglycemic effect of the plant extract also suggests their ability to stimulate more insulin secretion from a surviving pancreatic β-cells or promote more glucose utilization by peripheral tissues of diabetic rats.

The liver has an important function in maintaining blood glucose homeostasis and the hormone insulin regulates the glucose metabolism in the liver. The reduced glycogen content observed in diabetic rats may be associated with increase in glycogen phosphorylase activity. The disturbed activities of carbohydrate

metabolizing enzymes in diabetic animals are probably due to insulin deficiency. Oral administration of FrABet and FrEBet restored the activities of glucose-6-phosphatase, glycogen phosphorylase, fructose-1, 6-bisphosphatase, glucose-6-phosphate dehydrogenase, hexokinase and glycogen content in the liver of diabetic rats suggest their capacity to correct the metabolic alterations of carbohydrate metabolizing pathway in diabetes mellitus, as evident by increased plasma insulin and liver glycogen content in rats treated with FrABet and FrEBet.

Measurement of glycosylated haemoglobin is useful to monitor the glycemic control mechanism in diabetes mellitus. The lowered glycosylated haemoglobin and increased total haemoglobin in rats treated with FrABet and FrEBet indicates that the extracts of *F. racemosa* improved the glycemic control mechanism in diabetic rats.

Alloxan can cause DNA strand breaks in pancreatic islet cells through the formation of reactive oxygen species such as hydrogenperoxide, superoxide and hydroxyl radicals. This can lead to pancreatic damage, which is in turn responsible for hyperglycemia seen in diabetic animals (Soto *et al*., 1994; Murata *et al*., 1998). Several reports have shown elevated lipid peroxidation and decline in non-enzymatic antioxidants and antioxidants enzymes in diabetic conditions (Szaleczky *et al*., 1999). Hyperglycemia results in over production of reactive oxygen species, due to glucose autoxidation, non-enzymatic glycation of proteins and subsequent oxidative degradation of glycated proteins (Saraswathi *et al*., 2004). Increased concentration of thiobarbituric acid reactive substances and lipid hydroperoxides were observed in liver and kidney tissues during diabetes (Takasu *et al*., 2000). Increased membrane rigidity, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity are seen if the process of free radicals induced lipidperoxidation is excessively generated in erythrocytes (Ceriello, 2000). Enhanced susceptibility of erythrocytes to free radical mediated lipidperoxidation has been shown in various pathological conditions including diabetes (Punitha and Manoharan, 2006; Punitha *et al*., 2006). It has been reported that increased level of plasma lipid peroxides in diabetic individuals is due to increase in peroxidative damage of membrane lipids (Sabu *et al*., 2002). Lipidperoxide mediated oxidative tissue damage has been implicated in the pathogenesis of type 1 and type 2 diabetes mellitus (Ruiz *et al*., 1999; Seghrouchni *et al*., 2002). Elevated lipidperoxidation in liver and kidney has been well demonstrated in alloxan induced diabetic rats (Takasu *et al*., 2000). Determination of plasma TBARS and lipidperoxides is considered as the most reliable marker of tissue damage in pathological conditions (West, 2000). Increased plasma TBARS and lipid hydroperoxides observed in diabetic rats could be due to overproduction and diffusion of lipidperoxidation byproducts from damaged pancreas, liver, kidney and erythrocytes.

An array of non-enzymatic antioxidants (vitamin-E, vitamin-C and reduced glutathione) and enzymatic antioxidants (SOD, CAT, GPx) are involved in scavenging toxic free radicals *in vivo*. Both enzymatic and nonenzymatic antioxidant defense system is significantly impaired in alloxan induced diabetic rats. Lowered activities of enzymatic antioxidants and reduced levels of non enzymatic antioxidants observed in plasma and tissues in alloxan induced diabetic rats as compared to control rats could be due to increased oxidative stress. Oral administration of FrABet and FrEBet to alloxan induced diabetic rats significantly improved the antioxidant defense mechanism as well as restored the lipidperoxidation process. Reduced levels of plasma and tissue lipidperoxidation and improved status of nonenzymatic antioxidants and enzymatic antioxidants activities observed in FrABet and FrEBet treated rats, revealed their free radical scavenging activity and role in protection of vital tissues from oxidative damage.

The present study thus demonstrated that *Ficus racemosa* bark extracts have potent shown glucose lowering effect, antilipidperoxidative (reduced TBARS and LHP levels) and antioxidant function (improved enzymatic and non enzymatic antioxidants) in alloxan induced diabetic rats. *Ficus racemosa* bark extracts can therefore be recommended as an alternative remedy for the treatment of diabetes mellitus and its complications. Further studies are needed to isolate and characterize bioactive antidiabetic and antioxidant principles from *Ficus racemosa* bark.

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