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HYPOGLYCEMIC EFFECT OF AQUEOUS FRUIT EXTRACT OF *FICUS BENGALENSIS* IN NORMAL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT
The aim of the present study was to evaluate the antidiabetic and ameliorative potential of aqueous fruit extract of *Ficus bengalensis* (FB) in streptozotocin (STZ) induced diabetic rats. The effect of oral administration of aqueous fruit extract of FB on blood glucose, change in body weight, carbohydrate metabolizing enzymes, total cholesterol and lipid peroxidation in liver of STZ -induced diabetic rats were studied. Treatment of aqueous fruit extract reduced the rise in blood glucose levels and improved the enzyme acitivities to near normal in diabetic rats.

INTRODUCTION

Diabetes mellitus a leading non communicable disease with multiple etiologies, affects more than 100 million people worldwide and is considered as one of the five leading causes of death in the world (Zimmet, 1999). Diabetes is characterized by an increased concentration of blood glucose due to derangement in carbohydrates metabolism and defective secretion of insulin (Pavana *et al.,* 2007). Several studies have proposed the mechanism for the role of free radicals in the pathogenesis of various diseases including diabetes (Paolisso *et al.,* 1993). According to Palanduz *et al.* (2001) diabetic complications are associated with over production of free radicals accumulation of lipid peroxidation by products. However, an array of enzymatic antioxidants superoxide dismutases (SOD), catalase (CAT) defense mechanism are involved in the protection of free radicals induced oxidative damage.

Many herbal medicines as single agents or in different oral formulations have been recommended for diabetes due to the fact that they are less toxic than oral hypoglycemic agents such as sulfonylureas, metformin etc (Chattopadhyay, 1993). The *Ficus bengalensis* Linn, (FB) commonly known as the banyan tree, is member of Moraceae family and its bark is used in ayurvedic medicine for the treatment of diabetes mellitus (Kirtikar and Basu, 1993). 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 (Satyavati *et al.,* 1976). Among them the fruit activity of FB is not having scientific studies with anti-diabetic activity.

In the present investigation studies on hypoglycemic activity was conducted on STZ induced diabetic rats were given treatment with or without aqueous extract of FB fruits and the effect and protection was studied mainly on the carbohydrates, lipid metabolism and antioxidant defense.

MATERIALS AND METHODS

Drugs and chemicals

STZ was purchased from sigma aldrich chemicals, Pvt., Ltd., Bangalore. All other chemicals and reagents used were of analytical grade.

Plant material

The fruits of FB were collected from the Agriculture College; Tirupati affiliated to Acharya N.G. Ranga Agriculture University and identified them with the help of a Botanist, Department of Botany, Sri Venkateswara University, Tirupati.

Preparation of fruit extract

After drying in the shade the fruits were made into powder. The fruit powder was soaked in the water in different glass jars and kept at room temp for 2 days and the extract was collected by filtration. The extract were distilled and concentrated under reduced pressure in rotavapour and finally freeze dried. These extract were future used for giving treatment to the control and diabetic rats.

Tissue homogenate preparation

Liver (250 mg) were sliced into pieces and homogenized in appropriate buffer in cold condition (pH 7.0) to give 20 % homogenate (w/v). The homogenate was centrifuged at 1000

rpm for 10 min at 4ºC in cold centrifuge. The supernatant was separated and used for various biochemical estimations.

Animal

Male albino wistar rats with 4 months age (body weight approx.160g) were used for the present study.

Induction of diabetes mellitus

Diabetes mellitus was induced in wister rats by singe intraperitoneal injection of STZ (50 mg/ kg) dissolved in 0.1 M citrate buffer (pH 4.5) after overnight fasting for 12h. The diabetes was assessed by determining the blood glucose concentration with in 48 h after injection of STZ.

Experimental design

In the experiment a total number of 60 rats (30 normal, 30 STZ- diabetic surviving rats) were used. The rats were divided into 6 groups of 10 rats each. Group-I: Normal rats; Group-II: Normal rats + FB (50 mg/kg); Group-III: Normal rats + FB (120 mg/kg); Group-IV: STZ – induced diabetic rats; Group-V: STZ – induced diabetic rats + FB (120 mg/kg); Group-VI: STZ – induced diabetic rats + FB (120 mg/kg); After the experimental period, all animals were sacrificed by cervical disorder and biochemical studies were conducted on liver of control and experimental animals in each group. Blood was drawn from tail of conscious rats and glucose was estimated. The body weights of all groups were recorded at an interval of one week till the completion of the experimental period (30 days).

Carbohydrate metabolizing enzymes

Hexokinase was assayed by the method of Brandstrup *et al.,* (1957). Glucose -6- phosphatase was assayed by the method of Koide and Oda (1959). Fructose 1, 6-bis phosphatase was assayed by the method of Gancedo and Gancedo (1971).

Estimation of total cholesterol

Total cholesterol in the tissues was estimated by the method described by Allain *et al.,* (1974). Cholesterol esters were hydrolyzed by cholesterol esterase to free cholesterol and free fatty acids. The free cholesterol produced and pre-existing ones were oxidized by cholesterol oxidase to cholest-4-en-3-one and H_2O_2. The formed H_2O_2 reacted with 4-aminoantipyrine and phenol in the presence of peroxidase to produce red colored quinoneimine dye. The intensity of color produced was proportional to the cholesterol concentration.

Assay of antioxidant enzymes

Superoxide dismutase (SOD, EC 1.15.1.1) in the erythrocytes and tissues were assayed by the method of Kakkar *et al.,* (1984). The assay is based on the inhibition of the formation of NADH -phenazinemethosulphate, nitroblue tetrazolium formazon. The reaction was initiated by the addition of NADH. After incubation for 90ºC, adding glacial acetic acid stopped the reaction. The color developed at the end of the reaction was extracted into n-butanol layer and measured at 520 nm. The activity of catalase (CAT, EC 1.11.1.6) in the erythrocytes and tissues was determined by the method of Sinha (1972). Dichromate in acetic acid was converted to perchromic acid and then to chromic acetate, when heated in the presence of H_2O_2. The chromic acetate formed was measured at 620 nm.

Statistical analysis

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 $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

Blood drawn from STZ diabetic rats to determine the effective dose on (50 and 120 mg/kg dw) blood glucose and change in body weight were shown in different groups (Table 1). In control sample the concentration of plasma glucose was 75 mg/dL. The induction of diabetic raised the glucose level to 277 mg/dL. Treatment with two different concentrations (50 mg and 120 mg of fruit extract with control samples) is able to maintain almost equal concentrations of glucose levels as observed in control. But in the case of diabetic rats treatment with low concentrations as well as high concentrations brought the glucose level to 120 and 115 mg/dL. There is a decrease in body weight during diabetes treatment of rats with fruit extract brought long duration diabetes from 20g in the body weight. In control animals after fruit extract treatment similar weight was noticed. The present study clearly reveals that the aqueous fruit extract produces the maximum reduction in blood glucose level as compared to the extract of aerial root or bark of FB (Sharma *et al.,* 2009). Decrease in body weight of diabetic rats is possible due to catabolism of fats and protein, even though the food intake is more in diabetic rats than control. Oral administration of FB fruit extract significantly improves body weight in diabetic rats. Rajkumar *et al.,* (1997) have reported that increased catabolic reactions leading to muscle wasting might also be the cause for the reduced weight gain by the diabetic rats.

Table 1: Effect of FB fruit extract on glucose and changes of body weight in control and STZ- diabetic rats

Group	Glucose (mg/dL)	Change in body wt.
Control	82 ± 8.1	+20.1 ± 5.1
Normal + FB (50 mg/kg bw)	80 ± 8.1	+20.1 ± 4.1
Normal + FB (120 mg/kg bw)	78 ± 7.4	+20.2 ± 5.2
Diabetic control	265 ±10.1	-28.0 ± 8.2
Diabetic + FB (50 mg/kg bw)	125 ±10.1	-18.5 ± 7.1
Diabetic + FB (120 mg/kg bw)	101 ± 10.2	-10.0 ± 7.1

Each value is mean ± SD for 6 rats in each group; a: $p < 0.05$ by comparison with normal rats; b: $p < 0.05$ by comparison with STZ diabetic rats; No significance

Blood glucose levels are regulated by the pathways utilizing and generating glucose. Hence the activities of some of the key enzymes of carbohydrate metabolism were estimated in liver of control and diabetic supplemented rats (Table 2). The activity of hepatic glycolytic enzyme hexokinase was decreased while the activity of hepatic Glucose 6-phosphatase, Fructose 1, 6-bis phosphatase were increased in diabetic rats as compared to the normal rats. Oral administration of 15 days showed a significant ($p < 0.05$) and improved the enzyme activities to near normal in diabetic rats. One of the key enzymes in the catabolism of glucose is hexokinase, which

Table 2: Effect of FB fruit extract on carbohydrate metabolizing enzyme levels in control and STZ–diabetic rats

Groups	Hexokinase (μ moles of glucose phosphorylated / hr /mg protein)	Glucose-6-phosphatase (μ mole of Pi liberated/min/mg protein)	Fructose-1,6-bisphosphatase (μ mole of Pi liberated/min/mg protein)
Normal	125.89 ± 9.65	16.79 ± 1.20	7.11 ± 0.45
Normal + FB (70 mg/kg bw)	119.45 ± 9.65 b	14.45 ± 1.11 b	7.05 ± 0.42 b
Normal + FB (130 mg/kg bw)	128.54 ± 9.12 b	13.24 ± 1.02 b	7.65 ± 0.59 b
Diabetic control	53.51 ± 4.69 a	39.52 ± 3.06 a	21.46 ± 1.54 a
Diabetic + FB (70 mg/kg bw)	121.54 ± 9.12 b	15.54 ± 1.12 b	7.62 ± 0.68 b
Diabetic + FB (130 mg/kg bw)	117.06 ± 8.75 ab	18.03 ± 1.32 ab	8.77 ± 0.52 b

Each value is mean ± SD for 6 rats in each group; a: $p < 0.05$ by comparison with normal rats; b: $p < 0.05$ by comparison with STZ diabetic rats; No significance

Table 3: Effect of FB fruit extract on total cholesterol in control and STZ –diabetic rats

Groups	Total cholesterol (mg/dL)
Normal	84.52 ± 6.38
Normal + FB (70 mg/kg bw)	79.24 ± 5.85 b
Normal + FB (130 mg/kg bw)	71.26 ± 5.42 b
Diabetic control	264.12 ± 21.28a
Diabetic + FB (70 mg/kg bw)	90.68 ± 6.78ab
Diabetic + FB (130 mg/kg bw)	105.63 ± 7.92b

Each value is mean ± SD for 6 rats in each group; a: $p < 0.05$ by comparison with normal rats; b: $p < 0.05$ by comparison with STZ diabetic rats; No significance.

Table 4: Effect of FB fruit extract on TBARS, SOD, CAT levels in control and STZ-diabetic rats

Groups	TBARS (n moles/ 100 g tissue)	SOD (units a / mg protein)	CAT(n moles/ 100 g tissue)
Normal	1.62 ± 0.12	9.62 ± 0.72	81.32 ± 6.18
Normal + FB (70 mg/kg bw)	1.72 ± 0.15 b	10.81 ± 0.81 b	84.96 ± 6.47 b
Normal + FB (130 mg/kg bw)	1.78 ± 0.12 b	10.92 ± 0.88 b	86.94 ± 6.65 b
Diabetic control	3.97 ± 0.31 a	4.09 ± 0.30 a	43.22 ± 3.30 a
Diabetic + FB (70 mg/kg bw)	1.81 ± 0.12 b	9.28 ± 0.71 b	78.92 ± 6.01 b
Diabetic + FB (130 mg/kg bw)	1.89 ± 0.16 ab	9.82 ± 0.76 b	82.74 ± 6.33 b

Each value is mean ± SD for 6 rats in each group; a: $p < 0.05$ by comparison with normal rats; b: $p < 0.05$ by comparison with STZ diabetic rats; No significance.

phosphorylates glucose and converts it into glucose-6-phosphate (Laakso *et al.,* 1995). Increased glucose-6-phosphatase activity in diabetic rats provides H^+, which binds with $NADP^+$ in the form of NADPH and enhances the synthesis of fats from carbohydrates (*i.e.* lipogenesis) (Bopanna *et al.,* 1997) and finally, contributes to increased levels of glucose in the blood.

Hyper cholesterolemia result in experimentally induced diabetic animal and human diabetes. Hence effects are being made to estimate the levels of total cholesterol in liver (Table 3). The diabetic rats are showed elevated levels of total cholesterol compared to the control samples in liver. Lipids play a vital role in the pathogenesis of diabetes mellitus and the most common lipid abnormalities in diabetes are hypercholesterolemia (Palumbo, 1998). Our results supported by Pushparaj *et al.,* (2000) in serum and tissues of STZ diabetic rats. Ravi *et al.,* (2004) also reported the increased levels of tissue lipids in STZ-induced diabetic rats.

Increased concentrations of TBARS are observed in liver during diabetes (Table 4). There was a significant increase in TBARS in diabetic rats as compared to normal rats. Oral administration of FB fruit extract for 15 days exhibited a significant on the parameters. Rajasekaran *et al.,* (2005) have also reported the increase lipid peroxide levels in diabetic rats. Enhanced TBARS and declined antioxidants observed in the erythrocytes of diabetic rats can therefore be attributed to increased biomembrane lipidperoxidation process and there by contributing to alterations in antioxidants status (Pavana *et al.,* 2007).

The increase in the level of ROS are controlled by various enzymatic defense mechanisms consisting of SOD and CAT as presented in Table 4, the activities of antioxidant enzymes such as SOD and CAT were lowered in the STZ-diabetic tissues of liver when compared to the normal tissues. Oral administration of FB fruit extract for 15 days significantly increased the SOD and CAT activities. Decreased activities of enzymatic antioxidants such as SOD have been well documented in STZ induced diabetic rats (Sugiura *et al.,* 2006). Individuals with reduced CAT activity suffer a hightened risk of developing diabetes (Rajasekharan *et al.,* 2005).

The aqueous extract treatment of FB has normalized the enzyme activities and maintained the normal levels of glucose and lipid metabolisms as in the case of control. The observed increase in antioxidant status and decline in TBARS concentration in FB extract treated diabetic rats suggests its potent antilipidperoxidative and antioxidative effects.

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