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ANTIHYPERGLYCEMIC AND ANTIHYPERLIPIDEMIC ACTIVITIES OF ETHANOLIC EXTRACTS OF *FICUS RACEMOSA* (L.) IN ALLOXAN INDUCED DIABETES MICE

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ABSTRACT

The aim of the investigation was to evaluate the hypoglycemic and hypolipidemic activity of ethanolic extracts of different parts from the plant *Ficus racemosa* (FR) in alloxan-induced diabetic mice. Diabetes was induced by a single dose of intraperitoneal injection of alloxan (100mg/kg) in Swiss Albino Mice. Metformin HCl (150mg/kg body weight, op.) was used as a standard antidiabetic agent. After oral administration of fruits (300 mg/kg) and bark extracts (300 mg/kg), changes in blood sugar levels, body weight and organ weight were measured for seven days. At the end of the experiment blood samples were analyzed for the estimation of total cholesterol (TC), triglyceride (TG), phospholipid levels and liver glycogen content. The result indicated that oral ingestion of FR extracts significantly reduced blood glucose levels in Group-DFF (50.45%) and Group-DFB (60.53%) when compared to Group-DC mice. Short-term treatment did not alter the body weight among the mice; however the liver weight to body weight ratios were significantly reduced in Group-DFF and Group-DFB. Administration of extracts greatly reduced the serum cholesterols, triglycerides, phospholipids levels and the glycogen content were significantly restored in Group-DFF and Group-DFB as compared to Group-DC mice. Our findings suggested that extracts from different parts of *Ficus racemosa* have glucose and lipid lowering efficacy and may have some beneficial effects in patients with diabetes.

Keywords: *Ficus racemosa*, fruits, barks, antihyperglycemic and antihyperlipidemic

INTRODUCTION

Diabetes is a global disease with huge adverse impacts on health and mortality particularly of cardiovascular disorders. Diabetes mellitus (DM) is the major clinical disorder affecting nearly 10% of the populations all over the world [1]. The prevalence of the DM is increasing rapidly in the developing countries than in the developed country. There are an estimated 246 million people with diabetes in the world, of whom about 80% reside in developing countries [2]. Patient with diabetes have an increased risk of coronary heart disease, peripheral vascular disease, strokes and may account for more than 65% death among people with diabetes [3, 4 and 5]. Multiple pathophysiologic mechanisms play a role in the risk of cardiovascular events in the metabolic syndrome including glucose intolerance, hyperglycemia, hypertension, dyslipidemia, atherosclerosis that are caused primarily by insulin resistance [6, 7]. In particular hyperglycemia may contribute to diabetic complications by altering vascular cellular metabolism, vascular matrix molecules and circulating lipoproteins. However, an abnormal lipid profile was found to a more significant risk factor than either hypertension or diabetes alone [8]. The burden of death and disability from diabetes and its related complications remain great due to their therapeutic failure as well as the potential for induction of hypoglycemia [9]. Traditional medicines are used to reduce blood glucose level as well as have beneficial effects on complication of diabetes [10].

Therefore, natural agents having hypoglycemic and hypolipidemic properties would be better for the management of diabetes.

Ficus racemosa (Linn.) belonging to the family (Moraceae), commonly known as jogyadumur (Bengali) or Country Fig, an evergreen 15-18 m height tree, grows commonly throughout the country [11] . Different parts of *Ficus racemosa* (bark and fruits) are used in folk medicine for the treatment of several diseases including diabetes mellitus [12]. The phytochemical studies on the plant *Ficus racemosa* revealed the presence of glycosides, gluanol acetate, beta-amyrin, lupeol, acetate, α- and β-amyrin, beta sitosterol, stigmasterol [11]. *Ficus racemosa* bark extract contains antioxidant and chemoprotective pricinples such as racemosic acid and phenolic glycosides [13]. Experimental studies have demonstrated that the *Ficus racemosa* extract has antibacterial, larvicidal, anti-pyretic, anti-inflammatory activity and antitussive activity [14- 18]. It shown to be effective against oxidative damage in rats and possesses hepatoprotective properties [19-20]. However, the effects of *Ficus racemosa* fruits and barks extracts on blood glucose and lipid levels in diabetic mice have not been reported. In the present study, we investigated the anti-hyperglycemic and antihyperlipidemic activities of the extracts from different parts of *Ficus racemosa* (FR) in alloxan induced diabetic mice.

MATERIALS AND METHODS

Plant materials: Fresh plants (fruits & barks) of *Ficus racemosa* Linn. were collected from Rajshahi in August 2014 and the plant authenticity was confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka. A voucher specimen no. 33131 is maintained in our laboratory for future reference.

Extraction: The collected plant (barks & fruits) were washed and sun dried under shadow for several days. The powdered plant leaves were extracted with 96% ethanol at room temperature. The bottle were kept at room temperature and allowed to stand for several 7-10 days with occasional shaking and stirring. The extracts thus obtained were filtered through cotton and then through filter paper (Whatman Filter Paper No. 1). The filtrate was defatted with petroleum ether for several times. Then, the defatted liquor was allowed to evaporate using rotary evaporator at temperature 40-45°C. Thus the highly concentrated crude extracts were obtained.

Drugs and chemicals: The active drug, Metformin hydrochloride was the generous gift samples from Square Pharmaceuticals Ltd; Pabna Bangladesh. Total cholesterol (TC) and triglyceride (TG) wet reagent diagnostic kits were in the products of Crescent diagnostic kits. Alloxan was purchased from Sisco Research Laboratories Pvt. Ltd. Mumbai, India. All other chemicals and solvents were of analytical grade.

Animals: Nine weeks old male Swiss Albino mice (weight, 25-28g) were purchased from ICDDRB, Dhaka, Bangladesh and housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%) with water *ad. libitum*. The animals used in this study were cared in according to guidelines of animal experiment.

Phytochemical screening: Phytochemical analysis was performed according to the standard methods described by Nayek and Pereira [21].

Anti-hyperglycemic test: Anti-hyperglycemic test was performed according to standard method. All mice were divided into four groups and each group comprised of five mice. Groups were Group-DC (Diabetic Control groups receiving vehicle 0.5% methyl cellulose), Group-DS (Diabetic Standard group mice received Metformin HCl, 150 mg/kg), Group-DFF and Group-DFF (Diabetic *Ficus* Fruit and Diabetic *Ficus* Barks group mice received ethanolic extracts of *Ficus* fruits and barks 300 mg/kg body weight, dissolved in 0.5% methyl cellulose, respectively). After fasting 16 hours, diabetes was induced to all groups by intra-peritoneal injection (IP) of alloxan monohydrate (100 mg/kg) dissolved in saline. After 72 hours, blood glucose levels were measured from tail-vein blood of all groups by Glucometer considered as 0 day and blood glucose level higher than 11.5 mmol/l considered as diabetic. 0.5% methyl cellulose, standard drug metformin and extracts (300 mg/kg) were administered once daily for seven days to respective mice groups. Blood glucose content was measured after 1st, 3rd and 7th days by Glucometer.

Blood and organs sampling: The body weight of mice of each group was measured before and after week-long antihyperglycemic tested with drugs. The mice were sacrificed by anesthetizing with pentobarbital (5mg/kg, i.p.), blood samples were withdrawn from aorta of heart using a syringe and kept into an EDTA containing tube. Heart, liver and kidney were excised and cleaned of the surrounding tissues. The organ weights were measured immediately and the organ weight to body weight ratio were calculated. Finally, the blood and tissue

samples were preserved in refrigerator at -40C for biochemical estimations.

Estimation of serum cholesterol, triglyceride and phospholipids in diabetic mice: Serum samples were obtained by centrifugation of blood at 4000 rpm for 10 minutes. The concentration of TC and TG were measured by UV-spectrophotometer, using wet reagent diagnostic kits according to the manufacturer's protocol. Total lipid was extracted from the liver and kidney tissue according to the method of Folch et al [22]. Phospholipids were estimated by the method of Bartlette by digestion with perchloric acid and the phosphorous liberated was estimated by the method of Fiske and Subbarow [23-24].

Estimation of glycogen concentration in liver of diabetic mice: The liver glycogen content was determined according to the method described by Tarnoky K. et. al. Briefly, it utilizes the o-toluidine-glucose coupling reaction for the estimation of glycogen after trichloroacetic acid (TCA) extraction, precipitation by alcohol and hydrolysis [25].

STATISTICAL ANALYSIS

The results were expressed as mean ± Standard Error of Mean (SEM). Statistical analysis was performed by using ANOVA followed by Tukey's test using Graph pad Prism Software version 5.03. P values <0.05, p<0.01 and p<0.001 were considered as statistically significant.

RESULTS

Table 1. Phytochemical screening test result of FR extracts

Plant Extrats	Alkaloids	Saponins	Flavonoids	Tanins	Triterpenoids
Ficus fruits extract	+	+	+	+	+
Ficus barks extract	+	+	+	+	+

(+) = presence, (-) =Absence

Antihyperglycemic effect of CS extracts in diabetic mice: Hypoglycemic test was performed with extract showed more improvement compared with Group-DC. After 7 days mice treated with extracts in Group-DFF and Group-DFB (300 mg/kg body weight), glucose level were significantly lowered 50.45% and 60.53% respectively showed in table 2.

Table 2. Anti hyperglycemic effect of FR extracts in diabetic mice

Group	0 day	1st day	3rd day	7th day
DC	19.53±1.4	18.5±2.1	18.8±2.1	20.75±2.5
DS	18.75±1.35	13.3±1.3	10.3±1.0*	5.7±1.1***
DFF	16.35±0.7	14.2±2.2	13.3±2.1	8.1±1.5*
DFB	18.75±2.5	15.7±1.1	11.1±1.2*	7.4.±1.6**

Values were expressed in Mean ± SEM. Control group received 0.5% Methyl cellulose and standard group received Metformin 150 mg/kg. *p<0.05, **p<0.01, and ***p<0.001 indicate significant changes compared with diabetic control.

Effect of FR extracts on body weight, organ weight (heart, liver and kidney) and organ weight to body weight ratio changes: Table 3 shows no significantly changes in the body weight among experimental animals after seven days treatment. The results revealed that heart weight to body weight (HW/BW) and kidney weight to body weight (KW/BW) ratio did not altered significantly in experimental mice compared to Group-DC. Although the liver weight to body weight (LW/BW) ratio were severely altered or increase in Group-DC, after treatment. It was significantly decreased in Group-DFF and Group-DFB compared to the Group-DC mice although total food intake was not different.

Table 3. Effect of FR extracts on body weight and body weight to organ weight ratio in diabetic mice.

Group	Initial Body weight (g)	Final Body weight (g)	HW/BW (g/kg)	LW/BW (g/kg)	KW/BW (g/kg)
DC	28±1.40	35.5±1.20	0.77±0.12	8.5±1.5	1.7±0.21
DS	32±1.7	27±2.12	0.31±0.5	4.11±0.11**	0.72±0.16
DFF	27.6±2.12	28.0±2.12	0.42±0.19	5.4±0.10*	0.87±0.54
DFB	30.56±2.8	29±0.70	0.44±0.07	5.2±0.10*	0.30±0.04

Values were expressed in Mean ± SEM. Control group received 0.5% Methyl cellulose and standard group received Metformin 150 mg/kg. *p<0.05 and **p<0.01 indicate significant changes compared with diabetic control.

Effects of FR extracts on total cholesterol, triglyceride and phospholipid in diabetic mice: Comparison of serum lipid contents in control group and experimental groups of mice are shown in table 4. Administration of FR extracts and metformin to diabetic mice a significant decrease ($p<0.05$) in the levels of total cholesterol, triglycerides, and phospholipids observed in Group-DS, Group-DFF and Group-DFB compared to Group-DC. Administration of FR extracts and metformin mice tend to bring the levels of hepatic lipids to near normal level.

Table 4. Levels of serum total cholesterol, triglycerides and Phospholipids in diabetic mice after treatment with extracts or drugs.

Group	Total Cholesterol mg/dl	Triglycerides mg/dl	Phospholipid mg/dl
DC	180±1.75	204±2.4	173.5 ± 13.4
DS	86.6±1.45*	85.56±2.52*	91.7 ± 8.5*
DFF	100.57±1.74*	101.76±2.11*	138.5 ± 6.4*
DFB	88.8± 2.44*	90.7±2.04*	128.1±1.8*

Values were expressed in Mean ± SEM. Control group received 0.5% Methyl cellulose and standard group received Metformin 150 mg/kg. *$p<0.05$ indicate significant changes compared with diabetic control group.

Effects of FR extracts on liver glycogen level in diabetic mice: In this study the level of glycogen in liver is increased in Group-DS, Group-DFF and Group-DFB compared to Group-DC. Treatment of diabetic mice with metformin and experimental groups significantly ($p<0.05$) improved the level of glycogen content compared to Group- DC as shown in figure 1.Although Group-DS gave more significant result than Group-DFF and Group-DFB.

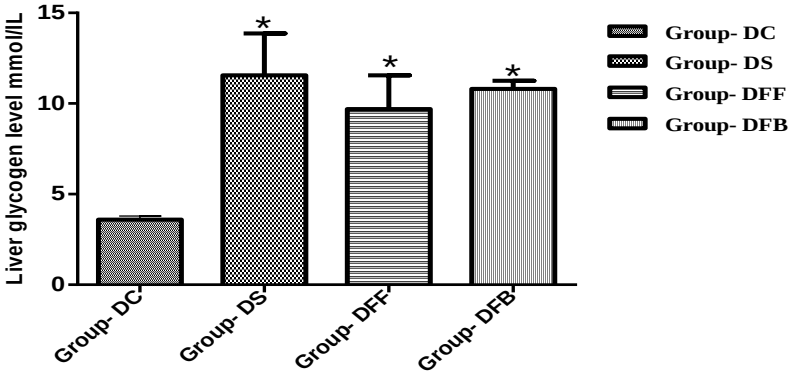

Figure 1: Effects of FR extracts on the liver glycogen level compared to diabetic control. Values were expressed in Mean ± Standard Error.*$p<0.05$ indicates significant changes compared with diabetic control.

DISCUSSION

The experiment showed that the antihyperglycemic experiment of extracts on diabetic mice showed lower blood glucose level (table 2). In this experiment, Group-DFF and Group-DFB has a significant decrease in blood glucose concentration 50.45% and 60.53% respectively after 7th day's treatment. Extracts may have the properties to stimulate ß-cell for the secretion of insulin and are most effective for controlling diabetes due to presence of hypoglycemic alkaloid, saponin and flavonoid (table-1). The extracts might be promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis [26]. Short-term treatment with extracts had no effect on body weight to organ weight ratio; however, it significantly lowered liver weight in Group-DFF and Group-DFB compared to Group-DC (table 3). In hyperglycemic mice there was a significant increased in lipids (total cholesterol and

triglycerides). The most common lipid abnormalities in diabetes are hypercholesterolemia and hypertriglyceridmia. Oral administration of FR extracts resulted in a significant reduction of serum lipids level in mice, viz total cholesterol and triglycerides. Flavonoids are known for their diverse activities including hypolipidemic and antioxidant activity [27]. FR extracts contain flavonoids and other different compounds such as saponin, tannin, triterpenes and alkaloids (table1). With respect lipid lowering capacity of this plant extract, it may be proposed that the constituents of the plant extracts may act as inhibitors for enzyme such as hydroxy-methyl-glutaryl-CoA reductase, which participates in de novo cholesterol biosynthesis (table 4) as has been suggested for some plants earlier [28]. The increase concentration of free fatty acid in liver and kidney may be due to lipid breakdown and this may cause increased generation of NADPH dependent microsomal lipid proxidation during diabetes. As a result liver and kidney phospholipids were increased in diabetic mice [29]. Administration of FR extracts significant decrease the level of tissue free fatty acids and phospholipids. Induction of diabetes with alloxan caused decrease in hepatic glycogen, which could be attributed to the decrease in the availability of the active form of enzyme glycogen synthetase probably because of low levels of insulin [30]. In this study, FR extracts restored the depressed hepatic glycogen levels possibly by increasing insulin (figure 1). Decreased activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animal resulting in depletion of liver and muscle glycogen content.

CONCLUSION

Based on results of present study, we conclude that the plant extracts of *Ficus racemosa* fruits and barks possess blood glucose and lipid lowering activities. However, further studies are needed to isolate active compounds responsible for these pharmacological activities and also necessary to examine underlying mechanism of antidiabetic and lipid lowering effects of *Ficus racemosa.*

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