HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC ACTIVITY OF METHANOLIC ROOT EXTRACT OF *FICUS RACEMOSA* IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

CHANDRASEKHAR VARMA[1*], J. FERNANDES[1], RONALD.F[1],

SUBRAMANYAM EVS[1]

1*.Chandrasekar Varma,M.Pharm,Research Scholar,Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences, Deralakatte, Mangalore – 574160, Karnataka, India.
E-mail:Chandrashakeralluri@hotmail.com/jennifer_pharma@yahoo.co.in

1. Dr.Jennifer Fernandes.M.Pharm,Ph.D,Reader
   Dr. Ronald Fernandes M.Sc.Ph.D,Reader
   Dr. E.V.S. Subramanyam.M.Pharm.Ph.D,Principal
   N.G.S.M. Institute of Pharmaceutical Sciences Deralakatte, Mangalore – 574160,

Summary

The study was undertaken to evaluate the hypoglycemic and antihyperglycemic activity of methanolic root extract of *Ficus racemosa* (Family: *Moraceae*) following oral administration (100, 200 and 400 mg/kg body weight) to normal, glucose-loaded and streptozocin-induced (50 mg/kg body weight, i.p) diabetic rats. The hypoglycemic/antihyperglycemic effect of extract was statistically significant with an oral dose of 400 mg/kg and was comparable to that of the effect produced by standard antidiabetic agent, glibenclamide 500 µg/kg. The increase in body weight and liver glycogen content in extract treated diabetic rats further supported the antihyperglycemic activity. In the oral glucose tolerance test, the extract increased the glucose tolerance.

Key words: Hypoglycemia, Antihyperglycemic activity, *Ficus racemosa*, Glucose tolerance test, Antidiabetic activity

Introduction

Diabetes mellitus is a chronic disorder characterized by disturbances in carbohydrate, protein and lipid metabolism and by complications like retinopathy, microanginopathy and nephropathy[1]. Diabetes mellitus is a global disease, found in all nations of the world and it is becoming a serious threat to the health of mankind and third killer of the human beings after cancer, cardiovascular and cerebrovascular diseases[2].Currently available synthetic oral hypoglycemic agents have side effects on prolonged use. Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus[3], investigations on hypoglycemic agents derived from medicinal plants have also gained momentum. Several investigations have been conducted and many plants have shown a positive activity. The utilization and awareness of the herbal medicines became popular because of minimal side effects, effectiveness and relatively low cost.

Ficus racemosa Linn., syn. *Ficus glomerata* Roxb. (Family: Moraceae), is a moderate to large-sized spreading tree widespread in moist land of India. It is commonly known as cluster fig and it's been used in Indian medicinal practice for the treatment of various ailments. The hypoglycemic and antihyperglycemic activity of fruits[4] and leaves[5] of the plant has been reported. The roots were used in the treatment of hydrophobia, diarrhea, dysentery, diabetes and pectoral complaints in traditional practice[6]. There is no scientific data available for antihyperglycemic and hypoglycemic activity of the roots of ficus racemosa. Hence the present study was under taken to ascertain the scientific basis for the use of this plant roots in the management of diabetes using streptozotocin-induced diabetic rats.

Materials and Methods

Plant material and preparation of extract

The roots of *Ficus racemosa* were collected from the local areas of Mangalore, identified and authenticated by Dr. Neoline J Pinto, Botanist, St.

Agnes College, Mangalore, India. A voucher specimen was deposited in the department herbarium for future reference.

The shade dried roots of *Ficus racemosa* were chopped and pulverized in an electric grinder. The powdered roots were macerated in methanol for four days with intermittent stirring. The mixture was filtered and the extract so obtained was distilled, evaporated on a water bath until dried extract was obtained and was stored in desicator for further usage.

Preliminary phytochemical screening

The methanolic roots extract of *Ficus racemosa* were subjected to preliminary phytochemical screening tests for the presence of flavanoides, tannins, saponins, alkaloids, carbohydrates and steroids[7].

Chemicals

All chemicals and reagents used were of analytical grade and obtained from LOBA chemicals, Mumbai, India. The kit for the estimation of blood glucose levels was obtained from Ranbaxy Diagnostics Pvt. Ltd., India. Glibenclamide was obtained as a gift sample by Hoechst Pharmaceuticals, Mumbai, India. Blood samples were analyzed by semiautoanalyser (Screen Master 3000, Mumbai, India).

Animals and Housing condition

Laboratory bred adult albino wistar rats of either sex weighing between 150-240 g were selected for the study. The animals were maintained under standard laboratory condition at 25±2°C relative humidity 50±15°C and normal 12:12 hour's light/dark cycle used for experiment. The experiment protocol has been approved by the Institutional Animal Ethics Committee of K.S. Hegde Medical Academy, Deralakatte, Mangalore, India (IAEC/017/2008-2009). The study was in accordance with the guidelines provided by committee for the purpose of control and supervision of experiments on animal (CPCSEA).

Acute toxicity study

The acute oral toxicity study (AOT) of methanolic roots extract of *Ficus racemosa* was carried out in female albino rats as per staircase method[8] and OECD guidelines 425[9].The animals were fasted 12hours prior to the dosing.Animals were administered orally with a single dose of extract suspended

in 0.6% CMC and observed for 2-3 hours for general,behavioural,neurological,autonomic profiles [10] and death for a period of 24hours and for 14 days after administration of the root extract.

Effect methanolic root extract of Ficus racemosa in normal rats[11]

The animals were fasted for 16 hours prior to the experiment, but allowed free access to water. Fasted rats were divided into four groups of six animals in each group. Group-I served as vehicle control which received distilled water p.o. The experimental groups (Group II, III and IV) received the methanolic root extract orally at different doses (100 mg/kg, 200 mg/kg and 400 mg/kg) respectively in an identical manner. Blood samples were collected from retro-orbital sinus at 0, 30, 60, 120, 180 and 240 min post dose. Serum glucose levels were estimated by GOD-POD method[12].

Oral glucose tolerance test (OGTT)

The OGTT[13] test was performed in overnight fasted normal rats. Rats were divided into four groups (n=6). Group-I served as vehicle control which received distilled water (p.o). Group II, III and IV received methanolic root extract (100 mg/kg, 200 mg/kg and 400 mg/kg) in an identical manner. After 30 min administration of methanolic roots extract of *Ficus racemosa* orally, the rats of all groups were orally loaded with 2 g/kg of glucose. Blood samples were collected from retro-orbital sinus at 0, 30, 60, 120 and 180 min after glucose loading. Serum was separated and glucose levels were measured immediately by GOD-POD method[12].

Effect of methanolic root extract of Ficus racemosa in streptozotocin induced diabetic rats [14]

Diabetes was induced in rats by the intraperitoneal injection of streptozotocin at a dose of 50mg/kg b.w. dissolved in freshly prepared citrate buffer (0.1M, pH 4.5) in a volume of 1 ml/kg b.w. Two days after the injection, the blood glucose levels were measured and the animals with blood glucose levels above 200 mg/dl were considered to be diabetic and were used in the experiment. In all the experiments, rats were fasted for 16 hrs prior to streptozotocin injection. Animals were divided into six groups of six rats each in a group. The test samples were administered for15 days through oral route.

Group I was maintained as diabetic control and was given distilled water. Groups II, III and IV were treated orally with different doses of methanolic root extract of Ficus racemosa-100 mg/kg, 200 mg/kg and 400 mg/kg respectively. Group V was treated with standard drug, Glibenclamide (0.05 mg/kg). Group VI was non diabetic rats and were kept as normal control. The above treatment was continued for 15 consecutive days once daily. Fasting blood glucose levels were estimated by GOD-POD method (prior to and on) on day 1, 4, 7, 10 and 15 respectively. Body weight of all the rats in each group was measured on the initial day and on the final day (i.e., day 15) of the study. On 15^{th} day the animals were sacrificed after blood collection under ether anesthesia and their livers were removed, homogenized and glycogen was estimated by anthrone method[15].

Statistical Analysis

The data were expressed as Mean ± SEM and analyzed by using one way analysis of variance (ANOVA), followed by post hoc Sheffe's test using SPSS computer software version 15. The values were considered significant when $p < 0.05$[16].

Results

Preliminary phytochemical screening of the methanolic roots extract of *Ficus racemosa* revealed the presence of flavanoides, tannins, saponins, alkaloids, carbohydrates and steroids. Different doses of methanolic roots extract of *Ficus racemosa* were screened for their oral toxicity. No mortality was recorded till 2000 mg/kg body weight of the extract.

Effect of MRF in normal rats

The MRF produced a dose-dependent hypoglycemia in normal rats. It produced maximum reduction in blood glucose level at a dose of 400 mg/kg body weight .The results are represented in Table No.1.

Effect MREF on oral glucose tolerance test

The effect of extract on oral glucose tolerance test is given in Table No. 2. Treatment with methanolic root extract to glucose loaded rats at different dose levels resulted in significant dose dependent reduction in blood glucose levels.

Effect of MREF in streptozotocin-induced diabetic rats

The STZ administration in experimental animals resulted in significant ($p < 0.05$) rise in blood glucose levels. The changes in body weights and fasting blood glucose levels, before and after treatment of the test drug in STZ induced diabetic animals is given in table No.3 & 4. Fasting blood glucose levels of untreated diabetic rats were significantly higher and the body weights were decreased than those in normal rats. In diabetic animals treated with extract showed significant dose dependent lowering of blood glucose levels and a significant increase in body weights ($p<0.05$). The liver glycogen levels in STZ induced diabetic rats were significantly lower than those in normal rats. Treatment with extract improved the liver glycogen significantly, as indicated by the higher levels of hepatic glycogen in the treated diabetic group compared to those in the untreated diabetic group ($p<0.05$).

Table 1: Effect of methanolic root extract of *Ficus racemosa* in normal rats (N=6)

Time (min)	**Blood Glucose Level (mg/dl)**			
	Control	**MREF-100 (mg/kg)**	**MREF-200 (mg/kg)**	**MRF-400 (mg/kg)**
0	86.06 ± 1.06	85.00 ± 1.15	83.50 ± 0.83	86.86 ± 0.85
30	85.35 ± 1.11	84.00 ± 1.02	83.25 ± 0.96	86.63 ± 0.74
60	85.83 ± 1.14	79.91 ± 0.82[a]	77.16 ± 1.08[a]	78.10 ± 0.72[a]
120	85.92 ± 1.09	74.31 ± 1.08[a]	68.53 ± 0.80[a]	66.28 ± 0.91[a]
180	85.93 ± 1.09	78.56 ± 1.05[a]	74.35 ± 0.75[a]	73.71 ± 1.18[a]
240	85.90 ± 1.14	83.56 ± 1.06	82.08 ± 0.97	82.65 ± 0.62

MREF: methanolic root extract of *Ficus racemosa*

[a] $P < 0.05$ when compared with the control group

Table 2 Effect of methanolic root extract of *Ficus racemosa* on oral glucose tolerance test in glucose loaded rats (N=6)

Time (min)	Blood Glucose Level (mg/dl)			
	Control	MRF-100 (mg/kg)	MRF-200 (mg/kg)	MRF-400 (mg/kg)
0	85.31 ± 1.16	84.95 ± 0.89	86.68 ± 1.35	86.83 ± 1.26
30	111.50 ± 1.3	107.43 ± 0.84	106.81 ± 1.37	99.70 ± 1.17[a]
60	100.43 ± 1.12	92.03 ± 0.91[a]	82.46 ± 1.22[a]	80.21 ± 1.10[a]
120	97.55 ± 1.09	88.01 ± 0.96[a]	83.50 ± 1.56[a]	82.26 ± 1.13[a]
180	95.60 ± 1.23	84.06 ± 0.92[a]	81.05 ± 1.53[a]	79.35 ± 1.23[a]

MREF: methanolic root extract of *Ficus racemosa*
[a] $P < 0.05$ when compared with the control group

Table 3 Effect of methanolic root extract of *Ficus racemosa* on body weight in streptozotocin induced diabetic rats (N=6)

Body Weight (g)				
Treatment	**Dose (mg/kg)**	**Initial day**	**Final day**	**% change in body weight**
Normal control	-	210.00 ± 5.16	230.50 ± 4.54	+9.93 ± 1.29[b,c]
Diabetic control	-	218.33 ± 6.00	192.16 ± 4.04	-12.06 ± 0.55[a,c]
MRF	100	208.33 ± 5.42	192.66 ± 8.34	-7.39 ± 1.74[a,b]
MRF	200	213.33 ± 4.94	198.16 ± 4.72	-7.10 ± 0.22[a,b]
MRF	400	210.00 ± 5.10	199.16 ± 4.79	-5.18 ± 0.25[a,b]
Glibenclamide	0.05	215.00 ± 6.70	207.16 ± 5.85	-3.66 ± 0.63[a,b]

MREF: methanolic root extract of *Ficus racemosa*
[a] $P < 0.05$ when compared with the normal control group
[b] $P < 0.05$ when compared with the diabetic control group
[c] $P < 0.05$ when compared with the glibenclamide group

Table 4: Effect of methanolic root extract of *Ficus racemosa* on Liver glycogen and Blood glucose level in streptozotocin induced rats (N=6)

			Blood Glucose Level (mg/dl)				
Treatment	**Dose (mg/kg)**	**Liver glycogen (mg/g)**	**1st day**	**4th day**	**7th day**	**10th day**	**15th day**
Normal control	---	43.98 ± 0.94[b,c]	85.5 ±0.95[b,c]	84.2 ±1.41[b,c]	86.25 ±0.99[b,c]	84.88 ±0.71[b,c]	85.66 ±0.91[b,c]
Diabetic control	---	10.21 ± 0.69[a,c]	336.33 ±4.8[a]	356.83 ±2.49[a,c]	362.16 ±3.01[a,c]	364.66 ±2.31[a,c]	372.83 ±2.58[a,c]
MRF	100	20.95 ±0.74[a,b,c]	334 ±2.88[a]	250.83 ±2.68[a,b]	241.16 ±2.25[a,b,c]	230 ±1.78[a,b,c]	205.33 ±2.34[a,b,c]
MRF	200	27.36 ± 0.73[a,b,c]	340.33 ±3.48[a]	247.5 ±3.28[a,b,c]	223.5 ±3.56[a,b]	205.33 ±3.75[a,b]	171.66 ±3.28[a,b,c]
MRF	400	33.10 ± 0.76[a,b,c]	339.5 ±2.95[a]	236 ±3.59[a,b,c]	203.16 ±2.35[a,b,c]	175.33 ±2.82[a,b,c]	151.83 ±2.18[a,b]
Glibenclamide	0.05	37.50 ± 0.88[a,b]	343.33 ±3.49[a]	263.66 ±3.46[a,b]	222 ±4.23[a,b]	193.5 ±4.36[a,b]	140.16 ±2.71[a,b]

MREF: methanolic root extract of *Ficus racemosa*

[a] $P < 0.05$ when compared with the normal control group

[b] $P < 0.05$ when compared with the diabetic control group

[c] $P < 0.05$ when compared with the glibenclamide group

Discussion

The present study has revealed the hypoglycemic and antihyperglycemic effects of the methanolic root extract of *Ficus racemosa* in normal and streptozotocin-induced diabetic rats respectively. Alloxan induced diabetic model resembles type I diabetes (insulin dependent diabetes mellitus) without significant insulin resistance whereas streptozotocin induced diabetic model exhibit reduced response to insulin in hepatic and peripheral tissues. Further, rats treated with streptozotocin display many of the features seen in human subjects with un-controlled diabetes mellitus[17].

When methanolic root extract was administered to glucose-loaded normal rats, hypoglycemia was observed in dose dependent manner. The effect of methanolic root extract was more significant with the dose of 400 mg/kg. From the oral glucose tolerance test, it is possible that the extract may act by direct stimulation of insulin secretion or increasing the glucose uptake. Our investigations also indicate the efficacy of the methanolic root extract of *Ficus racemosa* in the maintenance of blood glucose levels in normal and streptozotocin-induced diabetic rats. The antidiabetic activity of methanolic root extract was compared to that of Glibenclamide -0.05 mg/kg.

Further, induction of diabetes with streptozotocin is associated with a characteristic loss of body weight, which is due to increased muscle wasting[18] and loss of tissue proteins[19]. Increase in body weight in extract treated diabetic rats with respect to diabetic control may be due to the protective effect of the extract in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control. On the other hand, an increase observed in liver glycogen levels in treated groups in comparison to diabetic control group may be because of the reactivation of glycogen synthase systems[20]. This suggests a possible way by which the extract exhibit antidiabetic activity is by improvement of glycogenesis process.

Our phytochemical investigation revealed the presence of flavanoids, tannins, saponins, alkaloids, carbohydrates and steroids in methanolic root extract.

Presence of wide range of constituents indicates the good efficacy of this plant in various disorders. Presence of flavanoides[21], tannins[22], saponins[23] and alkaloids[24] are well known for their antidiabetic activities by different mechanisms. As the diabetes is a complex multifactorial disorder, the presence of wide range of constituents may contribute to the antidiabetic activity of methanolic root extract of *Ficus racemosa.*

From the results obtained in the current investigation, it may be concluded that methanolic root extract of *Ficus racemosa* possess significant antidiabetic activity and it might be help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of antidiabetic drugs.

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