ANTIHYPERGLYCAEMIC ACTIVITY OF PETROLEUM ETHER EXTRACT OF *FICUS RACEMOSA* FRUITS IN ALLOXAN INDUCED DIABETIC MICE.

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Summary

Ficus racemosa Linn. (family: Moraceae) commonly known as 'cluster fig' widely used in Indian folk medicine for the treatment of diabetes mellitus. In the present study, petroleum ether extract of *Ficus racemosa* (called as FRPE) was administered three doses (i.e.100, 200 and 400 mg/kg, p.o) to alloxan (70 mg/kg, i. v.) induced diabetic mice and serum glucose levels and body weights were measured. Oral glucose tolerance test (OGTT) was carried out after administration of FRPE in nondiabetic and diabetic mice previously loaded with (2.5 g/kg, p.o.) glucose. In acute oral toxicity (AOT 425) study, administration of FRPE no mortality upto 5000 mg/kg was observed. The onset of reduction of serum glucose was observed at 2 h (99.09 mg/dl), peak at 6 h (202.35 mg/dl) but antihyperglycaemic effect waned at 24 h. In subacute study, maximum reduction in serum glucose was observed: (257.82 mg/dl) at the dose of 200 mg/kg on 35th day. FRPE prevented further loss of body weight. The FRPE (200 mg/kg) showed increased glucose threshold in nondiabetic as well as diabetic mice. These results suggest that FRPE (200 mg/kg) showed antihyperglycaemic activity in alloxan induced diabetic mice.

Keywords: *Ficus racemosa*, Alloxan, Antihyperglycaemic, Body weight, Oral glucose tolerance test.

Introduction

Diabetic mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism. The worldwide survey reported that the diabetes mellitus is affecting nearly 10% of the population (1). Currently available synthetic oral hypoglycemic agents have side effects on prolonged use (2). The patients are using herbal medicines which have less side effects, easy availability and economic for them (3).One such plant mentioned to have antihyperglycaemic activity in Ayurvedic literature, *Ficus racemosa* Linn. Botanical synonyms *Ficus glomerata* Roxb. (Family: Moraceae) is a large deciduous tree distributed throughout India, particularly in evergreen forests, moist localities (4, 5). The fruits are considered astringent, stomachic and carminative. The tribal of Chotanagpur use *Ficus glomerata* in treatment for diabetes Petroleum ether extract of *Ficus glomerata* fruits showed significant hypoglycemic activity in rats (6). Hence, the objective of present investigation was to study the antihyperglycaemic activity of FRPE fruits in alloxan induced diabetic mice.

Material and Methods

Collection and authentication of plant

The fruits of *F.racemosa* were collected from the local area of Pune in Maharashtra state and were authenticated by Dr. A. M. Mujumdar, Department of Botany, at Agharkar Research Institute, Pune and voucher specimen was deposited at that Institute.

Drugs and Chemicals

Glyburide (Ranbaxy Pharma. Ltd. India), alloxan monohydrate (Spectrochem, India), glucose estimation kit (Accurex Biomedical Pvt. Ltd., India) and d-glucose (S.D. Fine-Chem. Ltd, India), petroleum ether 60^0-80^0 (Merck, Mumbai, India) and tween 80 (Research Lab., India) were purchased from respective vendors.

Extraction and preparation of FRPE

The fruits of *F.racemosa* were dried in shade and powdered in grinder. The air-dried powder were subjected to hot continuous extraction with petroleum ether (60^0-80^0) in a soxhlet extractor and filtered. The filtrate was evaporated in room temperature and the extract concentrated on a water bath to a dry residue. The % yield of petroleum ether extract was 17.3% w/w. The FRPE was suspended

in 5% tween 80 in distilled water to prepare the drug solution of concentration of 100 mg / ml and used for pharmacological studies.

Experimental animals

Swiss albino mice (25-30 g) of either sex were purchased from the National Toxicology Centre, Pune, India and used for the study. Animals were housed under standard condition of temperature 25 ± 1^0C and relative humidity of 45% to 55% under 12-h light: 12-h dark cycle. The animals had free access to food pellets (Chakan Oil Mills, Pune, India), and water was given *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Poona College of Pharmacy, Pune, India.

Acute oral toxicity studies

Healthy adult Swiss albino mice of either sex weighing between 18 to 23 g were subjected to acute toxicity studies as per guidelines (AOT no. 425) suggested by the Organization for Economic Cooperation and Development (7). The mice were observed continuously for 2 h for behavioral, neurological and autonomic profiles for any lethality or death for the next 48 h.

Induction of experimental diabetes

Diabetes was induced in mice by a single intravenous injection of aqueous alloxan monohydrate (70 mg /kg i.v.). After 48 h, the animals showing serum glucose levels above 200 mg /dl (diabetic) were selected for the study (8). All the animals were allowed free access to water and pellet diet.

Collection of blood and determination of serum glucose

Blood samples were collected by retro-orbital puncture (ROP) technique. The collected blood samples were analyzed for glucose levels by the glucose oxidase peroxidase (GOD/POD) method (9) and serum glucose levels were expressed in mg/dl.

Effect of FRPE on serum glucose in alloxan-induced diabetic mice

The diabetic mice of either sex were divided into five groups (n =6), viz.: group I-vehicle (5% tween 80, 10 ml/kg); group II-glyburide (10 mg/kg); group III-FRPE (100 mg/kg); group IV-FRPE (200 mg/kg); group V-FRPE (400 mg/kg). All drugs were given orally. The acute study involved estimation of serum glucose at 0, 2, 4, 6 and 24 h after drug administration (10).

The subacute study involved repeated administration of drug for 28 days at prefixed times and serum glucose levels were estimated on the 7th, 14th, 21st and 28th days. At the end of 28 days the drug administration was stopped and a

rest period of 7 days was given to the animals to study effect of drug treatment on blood glucose after 7 days i.e. on 35th bdays. The data were represented as mean serum glucose level and standard error of mean (SEM). The mice were weighed daily during the study period of 35 days, and their body weights were noted and presented as mean change in body weights.

Effect of FRPE on oral glucose tolerance test (OGTT) in normal and diabetic mice

The animals were fasted overnight before commencing the experiment. Nondiabetic and diabetic mice were divided into five groups (n = 6), viz.: group I-d-glucose (2.5 g/kg); group II-glyburide (10 mg/kg); group III-FRPE (100 mg/kg); group IV-FRPE (200 mg/kg); group V-FRPE (400 mg/kg). The animals were loaded with d-glucose (2.5 g/kg) solution after half an hour of glyburide and FRPE administration. Serum glucose levels were estimated prior to glyburide and FRPE administration and at 30, 60, and 120 min after glucose loading.

Statistical analysis

The results are expressed as mean ± S.E.M. and statistical analysis was carried out by One Way ANOVA followed by *post hoc* Tukey's test (11).

Results

In acute oral toxicity study, FRPE was safe upto a dose level of 5000 mg/kg of body weight. No lethality or any toxic reactions were found upto the end of the study period.

In acute study, FRPE (100,200 and 400 mg/kg) as well as glyburide (10 mg/kg) showed significant reduction of serum glucose levels at 2, 4, and 6 h. The onset of reduction of serum glucose of FRPE (100, 200 and 400 mg/kg) was observed at 2 h (94.75, 99.09 and 79.04 mg/dl respectively), peak effect at 6 h (174.69, 202.35 and 138.13 mg/dl respectively) but effect was waned at 24 h. The onset of antihyperglycaemic effect of glyburide was at 2 h (90.26 mg/dl), the peak effect was at 6 h (216.76 mg/dl) (Table 1).

Table 1: Effect of FRPE on serum glucose level in alloxan-induced diabetic mice (acute study).

Treatment (mg/kg.p.o.)	Mean Fasting Glucose Level (mg/ dl) ± SEM				
	0 h	2 h	4 h	6 h	24 h
Vehicle	436.45± 15.04	441.65± 11.51	450.70± 17.76	455.36± 21.52	458.08± 18.68
Glyburide (10)	441.57± 6.80	351.31± 19.84**	311.82± 23.70***	224.81± 22.97***	376.01± 26.38
FRPE (100)	460.02± 13.93	365.27± 11.70*	339.23± 18.45**	285.33± 20.14***	397.24± 22.44
FRPE (200)	453.00± 16.59	353.91± 12.17**	325.82± 16.09***	250.65± 18.11***	379.32± 20.00
FRPE (400)	457.27± 18.52	378.23± 16.11	356.21± 17.91*	319.14± 19.09***	414.15± 22.07

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group (5% Tween 80, 10 ml/kg).

In the subacute study, repeated administration (once a day for 28 days) of the FRPE as well as glyburide causes significantly ($P < 0.001$) reduction in the serum glucose level as compared with vehicle treated group. Maximum reduction in serum glucose level was observed (235.73, 257.82 and 189.10 mg/dl respectively) on 35th day in the diabetic mice treated with FRPE (100, 200 and 400 mg/kg). Glyburide treated animals showed maximum reduction in serum glucose level (274.80 mg/dl) on 35th day (Table 2).

Table 2: Effect of FRPE on serum glucose level in alloxan-induced diabetic mice (subacute study).

Treatment (mg/kg.)	Mean Fasting Glucose Level (mg/ dl) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Vehicle	436.45± 15.04	468.46± 17.18	475.33± 16.81	481.71± 16.27	495.25± 18.57	511.24± 17.80
Glyburide (10)	441.57± 06.80^{ns}	340.92± 19.19	292.68± 29.42	234.71± 32.21	181.77± 25.95	166.77± 25.99
FRPE (100)	460.02± 13.93 ^{ns}	350.16± 20.06	327.26± 21.29	295.27± 23.05	242.22± 19.89	224.29± 17.73
FRPE (200)	453.00± 16.59 ^{ns}	344.26± 19.01	306.24± 21.26	263.07± 22.09	217.21± 20.19	195.18± 18.56
FRPE (400)	457.27± 18.52 ^{ns}	368.32± 20.02**	349.26± 19.32	322.13± 18.73	294.14± 21.09	268.17± 20.27

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, ns- not significant, **P<0.01.All other values are significant (P<0.001) as compared with vehicle-treated group (5% Tween 80, 10 ml/kg).

Body weight of vehicle treated and FRPE (100 and 400 mg/kg) diabetic mice decreased during study period. FRPE (200 mg/kg) and glyburide (10 mg/kg) prevented further loss of body weight in diabetic mice. On the other hand, mice gained body weight which indicated beneficial effect of FRPE. (Table 3).

In oral glucose tolerance test, FRPE (200 mg/kg) produced significant (P<0.001) increase in glucose threshold, 60 min and 30 min post glucose loading in nondiabetic (Table 4) as well as diabetic (Table 5) mice respectively.

These results suggest that FRPE showed antihyperglycaemic activity in alloxan induced diabetic mice.

Table 3: Effect of FRPE on body weight in alloxan-induced diabetic mice.

Treatment (mg/kg.)	Mean Body Weight (g) ± SEM					
	Day 0	Day 7	Day 14	Day 21	Day 28	After day 7 rest period
Vehicle	30.66± 1.22	26.16± 0.90	24.16± 1.47	23.00± 1.23	20.33± 2.21	20.00± 2.29
Glyburide (10)	29.00± 0.68 ns	28.00± 0.81	30.00± 1.23	29.00± 1.41	30.00± 1.29	30.00± 1.36
FRPE(100)	32.16± 1.83 ns	31.33± 0.71**	30.66± 1.43	30.16± 1.77	29.00± 2.01	27.83± 2.12
FRPE(200)	29.33± 1.76 ns	30.50± 0.76*	30.83± 1.55	30.83± 2.02	30.50± 2.23	30.50± 2.23
FRPE(400)	33.00± 1.63 ns	31.50± 0.88**	31.00± 1.43*	33.33± 2.04	28.83± 2.40	27.66± 2.30

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, ns- not significant, *P<0.05,**P<0.01.All other values are not significant as compared with vehicle-treated group (5% Tween 80, 10 ml/kg).

Table 4: Effect of FRPE on oral glucose tolerance test (OGTT) in normal mice.

Treatment (mg/kg. p.o.)	Mean Fasting Glucose Level (mg/ dl) ± SEM				
	Before glucose	0 min	30 min	60 min	120 min
Vehicle	123.06± 11.16	323.26± 13.76	257.31± 10.09	208.02± 9.36	149.70± 10.12
Glyburide (10)	118.88± 12.03	300.92± 16.40	181.74± 9.59***	152.56± 11.67***	169.54± 10.78
FRPE (100)	108.21± 4.83	331.86± 19.16	191.78± 13.9**1	143.84± 12.02*	127.95± 11.09
FRPE (200)	110.79± 5.12	325.61± 17.80	180.46± 11.31***	129.19± 10.21***	137.99± 12.18
FRPE (400)	113.00± 6.18	329.05± 20.73	209.48± 15.12	162.61± 13.14	138.37± 14.43

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one-way ANOVA followed by Tukey's test using Graphpad Instat software, *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle-treated group (5% Tween 80, 10 ml/kg).

Table 5: Effect of FRPE on oral glucose tolerance test (OGTT) in diabetic mice.

Treatment (mg/kg. p.o.)	Mean Fasting Glucose Level (mg/ dl) ± SEM				
	Before glucose	0 min	30 min	60 min	120 min
Vehicle	399.82± 17.44	500.14± 13.37	429.12± 13.41	391.17± 9.48	493.23± 10.51
Glyburide (10)	452.29± 20.87	514.19± 13.06	326.77± 15.77***	314.61± 7.28**	437.78± 21.20
FRPE (100)	470.71± 17.85	507.82± 15.86	344.81± 13.43**	309.78± 12.44***	440.70± 16.19
FRPE (200)	464.87± 19.22	517.23± 17.91	330.80± 15.08***	342.93± 14.31	459.86± 16.95
FRPE (400)	478.43± 18.90	529.29± 16.79	366.05± 14.32	334.95± 13.18	427.01± 15.95

Values are mean ± S.E.M., n = 6 in each group, data were analyzed by one- way ANOVA followed by Tukey's test using Graphpad Instat software, **P<0.01, ***P<0.001 as compared with vehicle-treated group (5% Tween 80, 10 ml/kg).

Discussion

Ficus racemosa is used in medicine for the treatment of diabetes. The hypoglycemic properties of the *F. racemosa* fruits have been reported (6). Fresh whole fruits are used as a source of dietary fibre, which exhibited more hypocholesterolemic activity than pure cellulose (12). Some bioactive components from fruits, stembark and latex of the plant reduced the blood glucose level in streptozotocin induced diabetic rats (13).

FRPE (100, 200 and 400 mg/kg) showed significant P<0.001) decrease in serum glucose level at 2, 4 and 6 h. Continuous treatment with FRPE (100, 200 and 400 mg/kg) for a period of 35 days showed a significant (P<0.001) decrease in the serum glucose level in diabetic mice. Maximum reduction of serum glucose level in acute and subacute occurred at the dose of 200 mg/kg. p.o. The FRPE showed short onset and short duration of antihyperglycaemic action.

Subacute treatment for 35 days with the FRPE in the treated doses brought about improvement in body weights indicating its beneficial effect in preventing loss of body weight in diabetic mice (14). The ability of FRPE to prevent body weight loss seems to be due to its ability to reduced hyperglycaemia.

FRPE significantly enhanced glucose utilization in OGTT in both nondiabetic and diabetic mice. From the data obtained OGTT, it is clear that administration of FRPE effectively prevented the increase in serum glucose level without causing a hypoglycaemic state. The effect may be due to restoration of the delayed insulin response. In this context, other medicinal plants, such as *Pleurotus pulmonarius* (10), *Cassia auriculata* (15) have been reported to possess similar effects.

Steroid containing plants known to exhibit antidiabetic activity include the bark of various species of Ficus (16). β sitosterol was confirmed in the petroleum ether extract of fruits of *Ficus racemosa* by TLC (17).

Flavonoids are potent antioxidant and known to modulate the activities of various enzyme due to their interaction with various biomolecules (18). Kameswararao et al (1997) reported that flavonoids, alkaloids, tannins and phenolics as bioactive antidiabetic principles (19).

The plants of *F. racemosa* have been reported to contain gluanol acetate, hetriacontane (20), β sitosterol, lupeol acetate (21). Preliminary phytochemical analysis indicated that, the fruit extracts of *Ficus racemosa* contain sterols, triterpenoids, flavonoids, glycosides, tannins and carbohydrates.

The antihyperglycaemic activity of FRPE may probably be due to the presence of several bioactive antidiabetic principals. It is thus apparent that FRPE possesses antihyperglycaemic activity.

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