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Research Article

Anti-Diabetic and Antihyperlipidemic Activity of *Ficus krishnae* L. in Alloxan Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, neuropathy and causing neurological disorders due to perturbation in utilization of glucose. In the present study diabetes was induced in albino rat models with alloxan monohydrate. Ethnomedicinal survey reveals that the herbal preparations of leaves of *Ficus krishnae* had been considered as effective economical and safe treatments for curing various diseases in Indian traditional system of medicine including diabtes. Therefore, the present study to investigate the anti-diabetic activity of petroleum ether extract of *Ficus krishnae Linn.* (PFK) in alloxan induced diabetes in albino wistar rats. Alloxan was administered as a single dose (120mg/kg, b.wt) to induce diabetes. Administration of pet ether extracts from leaves of *Ficus krishnae Linn.* (200 &400mg/kg body weight/day) for 14 days, to alloxan-induced diabetic rats. The fasting blood sugar levels and serum biochemical analysis in alloxan-induced diabetic rats were investigated. The results suggest that the administration of *Ficus krishnae* have a anti-diabetic effect in alloxan induced diabetic rats and their effect was equivalent to that of reference drug glibenclamide.

Keywords: *Ficus krishnae Linn.*, Antidiabetic Activity, Alloxan Induced Diabetes, Total Cholesterol, HDL, LDL, VLDL and Triglycerides

INTRODUCTION

Diabetes mellitus is a global burden as its incidence is considered to be high (4–5%) all over the world [1]. However, quest for the development of more effective antidiabetic agents is being pursued relentlessly. Recently, herbal products have started gaining importance as complementary and alternative medicine to treat diabetic mellitus [2].

Ficus krishnae Linn. (Family-Moraceae) is a fast growing ever green tree grows up to 10 meters. *Ficus krishnae* is one among them in which it has been used in ancient folklore medicine. It is an unusual variant of the banyan. Stem bark and leaves are used for diabetes [3]. Information based on ethnomedicinal survey reveals that the herbal preparations of leaves of *Ficus krishnae* had been considered as effective economical and safe treatments for curing various diseases in Indian traditional system of medicine. In the present study we attempt to evaluate the anti diabetic potential of PFK in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

Ficus krishnae L. were collected from Tirumala hills, Tirupathi, Chittoor (dist), Andhra Pradesh, India. The taxonomical identification of the plant was done by Dr. K. Madhava chetty, Dept of Botany, S.V University. The voucher specimen was preserved in the department of Pharmacognosy laboratory of Sree Vidyanikethan College of Pharmacy for future reference.

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Preparation of plant extract

The collected plant leaves was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 120g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment. The percentage yield of prepared extract was around 10%w/w.

ANTI-DIABETIC ACTIVITY

The method of *Dash et al., 2001* [4] was followed. The test samples were suspended in 2%v/v Tween 80 in distilled water. Glibenclamide (2.5 mg/kg) was used as reference control during the study. All the test samples were administered through oral route.

Single dose study
In normoglycemic rats

The rats were fasted for 18 h, but were allowed free access to water before and throughout the duration of experiment. At the end of the fasting period, taken as zero time (0 h), blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. Plasma was separated following centrifugation the glucose was estimated by using Glucose estimation kit from 'One touch ultra', Life Scan, Johnson and Johnson, Milpitas, C.A., U.S.A. The normal rats were then divided into three groups of six rats each. Groups I and II received the test extract at a dose of 200 and 400 mg/kg, respectively, through oral route. Group III received glibenclamide (2.5 mg/kg) and served as reference control. All the test samples were administered in a similar manner. Blood glucose levels were examined after 1, 3, 5, 7 and 24 hrs of administration of single dose of test samples.

In Alloxan induced diabetic rats

The acclimatized rats were kept fasting for 24 hrs with water *ad libitum* and injected intraperitoneally a dose of 120 mg/kg of Alloxan monohydrate in normal saline. After 1 hr, the rats were provided feed *ad libitum*. The blood glucose level was checked before Alloxanisation and 24 h after Alloxanisation as above.

EXPERIMENTAL DESIGN

Rats were considered diabetic when the blood glucose level was raised beyond 200 mg/100 ml of blood. This condition *was* observed at the end of 48 hrs after Alloxanisation. The rats were segregated into four groups of six rats in each.

Group I - Diabetic Control and rats received only vehicle (2 ml/kg p.o) 2% v/v Tween 80.

Group II – Rats received Petroleum Ether Extract of *Ficus krishnae* I (200 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group III - Rats received Petroleum Ether Extract of *Ficus krishnae* II (400 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group IV – Rats received Glibenclamide (2.5 mg/kg p.o) suspended in 2% v/v Tween 80 solution.

MULTIDOSE STUDY
In Alloxan induced diabetic rats

The selected rats were treated with similar test samples as above, but the blood glucose level was measured on 0, 3, 7, and 14 days of treatment.

ESTIMATION OF LIPID PROFILE

Estimation of Lipid profile such as Total Cholesterol, Triglycerides, HDL, LDL, VLDL and serum glucose level [5].

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

RESULTS
Effect of PFK on blood glucose level

There were observable changes in BGL and lipid profile of treated and untreated rats. Treatment of diabetic rats with petroleum ether extract of *Ficus krishnae* and Glibenclamide significantly decreased the BGL compared to untreated diabetic rats. Dose dependent reduction in BGL, TC, TG and Lipid levels was observed in Alloxan induced diabetic rats treated with petroleum ether extract of *Ficus krishnae*.

Single dose study

After single dose of the PFK (200 or 400 mg/kg, oral) on the Alloxan induced diabetic rats, there was a

significant reduction (P>0.01) in BGL of the diabetic rats with in the period of acute study which was seven hours compared to the control. The effect was significant like the standard drug, Glibenclamide. PFK at the dose of 400 mg/kg body weight exhibited better BGL reduction (70.74%) than 200 mg/kg body weight (68.7%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (71.42%) at the same period (Table: 7.1)

Multidose study

During prolonged study (14 days), the PFK (200 or 400 mg/kg) produced a significant (P>0.01) in BGL of the diabetic rats compared to control. PFK at the dose of 400 mg/kg body weight exhibited better BGL reduction (74.39%) than 200 mg/kg body weight (65.74%) and that produced by the standard drug, Glibenclamide 2.5mg/kg (75.77%) at the same period. (Table: 7.2).

Serum lipid profile

Beneficial effects of PFK on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data (Table 7.3). Compared with the control values, the PFK (200 or 400 mg/kg) groups showed significant reduction (P>0.01) in the serum levels of total cholesterol, triglycerides, HDL, LDL and VLDL levels.

Table 7.1: Effect of *Ficus krishnae* on blood glucose levels of Alloxan induced diabetic rats after a single dose

Groups	Drugs	Dose	Initial	1hr	3hr	5hr	7hr	24hr
I	Diabetic control	2% Tween 80 w/v soln	267.40±1.46	278±2.47	285±1.37	282±1.19	271.5±2.63	284±1.32
II	Diabetic control + PFK	200 mg/kg	279.67±2.16 ^{nsb}	216±1.69 ^{nsb}	196.33±1.49 ^{**b}	152.50±2.20 ^{**b}	104.7±2.16 ^{*b}	91.83±1.2 ^{**b}
III	Diabetic control + PFK	400 mg/kg	280.83±1.4 ^{nsb}	22483±3.72 ^{*b}	194.16±2.08** ^{b}	149.12±1.24** ^{b}	109±2.94 ^{**b}	86.17±2.17 ^{**b}
IV	Diabetic control + standard	Glibenclamide (2.5 mg/kg)	281.67±1.94 ^{nsa}	205±1.37 ^{**a}	151.83±1.22 ^{**a}	139.17±1.70 ^{**a}	100.67±2.49 ^{**a}	84.67±1.89 ^{**a}

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. diabetic + PFK - 200 & 400 treated rats compared with diabetic control rats. **b**. diabetic + Glibenclamide treated rats compared with diabetic control rats.

Table 7.2: Effect of *Ficus krishnae* on blood glucose levels of Alloxan induced diabetic rats after a prolonged treatment

Groups	Drugs	Dose	Initial	Third day	Fifth day	Seventh day	Fourteenth day
I	Diabetic control	2% Tween 80 w/v soln	267.40±1.46	269±1.07	268±1.41	269.33±1.58	269.17±1.97
II	Diabetic control + PFK	200 mg/kg	279.67±2.16 ^{nsb}	209±1.21 ^{nsb}	159.5±1.20 ^{**b}	127±2.67 ^{**b}	99±2.42 ^{**b}
III	Diabetic control + PFK	400 mg/kg	280.83±1.4 ^{nsb}	162.16±2.19 ^{**b}	97.83±2.88 ^{**b}	84±2.25 ^{**b}	77±3.28 ^{**b}
IV	Diabetic control + standard	Glibenclamide (2.5 mg/kg)	281.67±1.94 ^{nsa}	78.33±3.10 ^{**a}	76.83±1.51 ^{**a}	76.67±1.25 ^{**a}	70.17±2.45 ^{**a}

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. diabetic + PFK - 200 & 400 treated rats compared with diabetic control rats. **b**. diabetic + Glibenclamide treated rats compared with diabetic control rats

Table 7.3: Effect of *Ficus krishnae* on Total Cholesterol and Triglycerides levels of Alloxan induced diabetic rats after a prolonged treatment

Groups	Drugs	Dose	Total Cholesterol	Triglycerides
Group I	Normal Control	2% Tween 80 w/v soln	72.80±2.40	74±2.07
Group II	Diabetic control	2% Tween 80 w/v soln	289.5± 2.21**[a]	194 ± 1.88**[a]
Group III	Diabetic control + PFK	200 mg/kg	138.66 ± 0.98*[b]	111.16 ± 0.87*[b]
Group IV	Diabetic control + PFK	400 mg/kg	93 ± 1.21**[b]	71.66 ± 1.17**[b]
Group V	Diabetic control + standard	Glibenclamide (2.5 mg/kg)	85.5 ± 0.99**[c]	68.33 ± 1.56**[c]

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. Normal control rats compared with diabetic control rats. **b**. diabetic + PFK - 200 & 400 treated rats compared with diabetic control rats. **c**. diabetic + Glibenclamide treated rats compared with diabetic control rats.

DISCUSSION

Evaluation of anti diabetic activity using Alloxan induced hyperglycemia model has been described by Dash *et al* [4]. The petroleum ether extract of *Ficus krishnae* (PFK) was screened to explore the scientific basis of its utility for correction of biochemical changes in Alloxan-induced diabetic rats. Despite the folk medicine use, so far, there have been no studies on its antidiabetic effect. However, presence of triterpenoids [6], which possess hypoglycemic and antihyperglycemic properties, has been reported in literature [7,8]. These compounds has been implicated in the anti diabetics activities of many plants [9].

Models of experimental diabetes that utilizes diabetogenic agent Alloxan induced blood glucoselevels higher than 250 mg/dL [10] which has been considered as severe diabetes. Diabetis mellitus is one of the most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes.

The possible mechanism of action of extract could be correlated with reminiscent effect of the hypoglycemic sulphonyl ureas, glibenclamide, that promote insulin secretion by closure of k+-ATP channels, membrane depolarization and stimulation of Ca 2+ influx , an initial key step in insulin secretion. In this context, number of other plants have been reported to have anti hyperglycemic and insulin stimulatory effects [11,12].

Several workers have shown that hyperglycemia and hyperlipidemia are the common characteristics of Alloxan-induced diabetes mellitus in experimental rats [13,14,15]. The maximum reduction (74.39%) in serum glucose levels was seen in PFK at the dose of 400 mg/kg. Thus, we could say that PFK had a beneficial effect on carbohydrate metabolism in diabetic rats. The hypoglycemic effect of PFK may be due to potentiating the insulin activity either by increasing the pancreatic secretion of insulin from cells of islets of langerhans or its release from bound insulin [16].

Dyslipidemia is a frequent complication noted in chemical induced diabetes [14,17,15] and presents a serious risk of vascular disease. In this study, we have also observed an increase in the concentration of TC and TG in alloxan induced diabetic rats. Hyperlipidemia is a recognized consequence of diabetes meliitus [18,10]. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of the glucose [19]. Regarding the mechanism of action PFK may enhance activity of enzymes involved in bile acid synthesis and its excretion and this may have decreased in serum cholesterol and triglycerides [20]. The decrease in serum TG level is an important finding because recent studies show that TG are independently related coronary heart disease.

Most of the hypolipidemic drugs do not decrease serum TG level, but PFK lowered it significantly since under normal condition, insulin activates the enzyme lipoprotein lipase and hydrolysis TG [21]. Plant extract reduces the serum TG of alloxan induced diabetic rats and may prevent the progression of CHD. Accumulation of TG is one of the risk factor in coronary heart disease (CHD). The total lipid profile in serum (total cholesterol, triglycerides) of the Alloxan induced diabetes rats treated with PFK (200 or 400 mg/kg, p.o) showed significant reduction, as compared to diabetic control rats. This suggests that PFK can prevent or be helpful in reducing the complications of lipid profile observed in some diabetics in

whom hyperglycemia and hypertriglyceremia coexist quite frequently. The strong anti-hyperglycemic effect of PFK could indirectly be related to beneficial action against the abnormal high concentration of serum lipids observed in diabetes rats.

The preliminary acute toxicity studies have revealed no visible signs or symptoms of toxicity of the PFK in normal rats. Therefore, the present studies have substantiated the folklore practice of *Ficus krishnae L.* for routine treatment of diabetes mellitus. In conclusion, the leaves of *Ficus krishnae L.* are a good candidate for alternative and/or complementary medicine in the management of diabetes mellitus.

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