HYPOGLYCEMIC ACTIVITY OF FICUS *GLOMERATA* 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. FICUS *GLOMERATA* Linn, has been claimed to possess antidiabetic properties by many investigators. The present study was undertaken to screen the hypoglycemic activity of ethanol extracts of leaves of F.Glomerata. The results showed that it has significant antihyperglycemic effect in experimental model of diabetes mellitus.

Keywords: Alloxan, Diabetes mellitus, Ficus glomerata, Blood glucose.

INTRODUCTION

Diabetes is a major degenerative disease in the world today (1), affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (2). Diabetes mellitus is also associated with long-term complications, including retinopathy, nephropathy, neuropathy and angiopathy and several others (3).

India has today become the diabetic capital of the world with over 20 million diabetics and this number is set to increase to 57 million by 2025 (4). Diabetes mellitus (DM) is a multifactorial disease which is characterized by hyperglycemia (5), lipoprotein abnormalities (6), raised basal metabolic rate (7), defect in reactive oxygen species scavenging enzymes (8) and high oxidative stress induced damage to pancreatic beta cells (9). Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when it's fatal complications are taken into account (10).

Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (11). There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents.

One such plant is *Ficus glomerata* which has been used in traditional system of medicine for treating liver diseases, diarrhoea, piles, asthma, leprosy and other ailments (12).

The fruits of *Ficus glomerata*, locally known as Gular have been used since olden times in the ethnomedicine for many varied medicinal purposes including as a remedy of diabetes mellitus (13).

In addition, this plant is considered to posses tonic, expectorant, emollient, stomachic and carminative properties (14). The sap extracted from trunk of the tree is also considered curative in diabetes. Moreover, the powdered seeds mixed with pure bee-honey are prescribed to the diabetics in the folklore medicine (15). The present research programme was aimed to investigate antidiabetic activities of leaf extract of *Ficus glomerata in* alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

The leaves of Ficus glomerata were collected in the month of April from the surrounding fields of Harapanahalli and authenticated by Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli.

The leaves were dried in shade at room temperature. The dried leaves were powdered by using grinder to coarse powder, packed into Soxhlet column and the extracted 70% ethanol for 24 hrs. The excess of solvent was removed using rotatory flash evaporator. The obtained crude extract was stored in airtight container in refrigerator below 10^0C for further studies.

Experimental Animals

Male albino rats of (180-210 g) were used throughout the experiments. The animals were procured from Sri Venkateshwara Enterprises, Bangalore. Before initiation of experiment, the rats were acclimatized for a period of 7 days. Standard environmental conditions such as temperature (26 ± 2^0C), relative humidity (45-55%) and 12 hrs dark/light cycle were maintained in the quarantine. All the animals were fed with rodent pellet diet (Gold mohr, Lipton India Ltd.) and water was allowed *ad-libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

Induction of diabetes

Alloxan (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone) is an oxygenated pyrimidine derivative

(16) and was originally isolated in 1818 by Brugnatelli and got its name in 1838 by Friedrich Wöhler and Justus von Liebig (17). Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan Diabetes") in these animals, with characteristics similar to type 1 diabetes in humans (Lenzen, 2008). Alloxan monohydrate was obtained from S.D. Fine, Mumbai and all the other chemicals used were of analytical grade and were acquired from commercial sources.

Anti-diabetic activity

Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan monohydrate (s.d. fine-chem. Ltd., Mumbai, India) in sterile saline. After 5 days of alloxan injection, the hyperglycemic rats (glucose level > 250 mg/dl) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. The treatment (p.o.) was started from the same day except normal control and diabetic control groups for a period of 10 days. During this period, animals in all groups had free access to standard diet and water. Body weight and blood glucose levels were estimated on 4^{th}, 7^{th} and 10^{th} day of the treatment. On the 10^{th} day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimations.

The various groups used in experiment

Group A - Served as normal control and did not receive any treatment.

Group B - Served as diabetic control and received alloxan monohydrate and vehicle (0.2 ml of 2% aqueous gum acacia)

Group C - Alloxan monohydrate + Glibenclamide (10 mg/kg, p.o.) and served as Standard.

Group D - Alloxan monohydrate + Ethanolic extract (100 mg/kg, p.o.)

Group E - Alloxan monohydrate + Ethanolic extract (250 mg/kg, p.o.)

Group F - Alloxan monohydrate + Ethanolic extract (500 mg/kg, p.o.)

RESULTS

Anti-diabetic study

Effect of *Ficus glomerata* leaf extract on fasting blood glucose level in diabetic rats (Table 1)

Ethanolic extract of *Ficus glomerata* leaves was subjected to anti-diabetic activity in rats where alloxan monohydrate (120 mg/kg b.w., i.p.) used as the diabetogenic agent.

A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats. Ethanolic extract of *Ficus glomerata* (at 250 and 500 mg/kg) exhibited a dose dependent significant anti-hyperglycemic activity on $4^{th,}$ 7^{th} and 10^{th} day post treatment. The extract dose of 100 mg/kg also caused reduction in blood glucose level but the results were found statistically insignificant. The antihyperglycemic effect of ethanol extract at was found less effective than the reference standard, Glibenclamide. Glibenclamide produced a significant reduction in blood glucose compare to diabetic control. The results are shown in the Table No. 1.

Table -1 Effect of *Ficus glomerata* leaf extract on fasting blood glucose level in alloxan induced diabetic rats

Animal: Albino Rats Alloxan: 120 mg/kg, i.p.

Extract: p.o.

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Basal value	4^{th} day	7^{th} day	10^{th} day
A	Normal Control	90.46 ± 3.80	92.82 ± 2.92	92.32 ± 1.73	88.29 ± 3.44
B	Diabetic Control (Vehicle)	293.8 ± 5.27	286.91 ± 5.05	291.8 ± 5.41	289.41 ± 9.75
C	Alloxan + glibenclamide (10 mg/kg)	285.86 ± 6.92	205.25 ± 7.06***	183.18 ± 6.35***	178.13 ± 6.20***
D	Alloxan + Ethanolic extract (100 mg/kg)	291.76 ± 4.79	277.76 ± 5.65	266.23 ± 8.19	255.42 ± 7.71
E	Alloxan + Ethanolic extract (250 mg/kg)	284.48 ± 5.32	258.23 ± 6.66*	255.85 ± 9.97**	252.06 ± 9.19**
F	Alloxan + Ethanolic extract (500 mg/kg)	287.48 ± 5.32	212.61 ± 5.07***	198.36 ± 3.52***	189.83 ± 3.31***

Values are Mean ± S.E.M; n=6; *P < 0.05, **P < 0.01 and ***P < 0.001 vs Diabetic Control

Table -2 Effect of *Ficus glomerata* leaf extract on biochemical parameters in alloxan induced diabetic rats

Group	Treatment	Serum Urea (mg / dl)	Serum Creatinine (mg/dl)	Serum Cholesterol (mg/dl)	Serum Protein (g/dl)
A	Normal Control	30.09 ± 1.09	0.56 ± 0.02	106.40 ± 2.06	6.17 ± 0.16
B	Diabetic Control (Vehicle)	62.93 ± 1.10	1.47 ± 0.04	179.80 ± 1.46	4.71 ± 0.95
C	Standard (Alloxan + glibenclamide 10 mg/kg)	33.97 ± 1.34***	0.64 ± 0.01***	121.9 ± 2.15***	6.10 ± 0.18***
D	Alloxan + Ethanolic extract (100 mg/kg)	59.30 ± 1.48	1.25 ± 0.02	170.4 ± 6.16	4.97 ± 0.13
E	Alloxan + Ethanolic extract (250 mg/kg)	44.08 ± 1.67***	0.86 ± 0.02***	145.03 ± 2.78***	5.60 ± 0.15**
F	Alloxan + Ethanolic extract (500 mg/kg)	37.33 ± 0.75***	0.74 ± 0.01***	125.65 ± 2.75***	5.87 ± 0.22***

Values are Mean ± S.E.M; n=6

**P < 0.01 and ***P < 0.001 vs Diabetic Control.

Table -3 Effect of *Ficus glomerata* leaf extract on body weight in alloxan induced diabetic rats

Animal: Albino Rats

Alloxan: 120 mg/kg, i.p.

Extract: p.o.

Group	Treatment	Body weight of the animal (g)			
		Initial	4th day	7th day	10th day
A	Normal Control	201.50 ± 2.75	203.0 ± 2.78	205.60 ± 2.80	209.90 ± 2.86
B	Diabetic Control (Vehicle)	203.50 ± 2.89	172.00 ± 2.59	158.00 ± 2.51	145.00 ± 1.72
C	Standard (Alloxan + glibenclamide 10 mg/kg)	206.50 ± 2.84	203.00 ± 2.64*	196.50 ± 2.02*	192.00 ± 1.90*
D	Alloxan + Ethanolic extract (100 mg/kg)	206.00 ± 2.79	178.00 ± 2.41	163.00 ± 2.32	152.00 ± 1.72
E	Alloxan + Ethanolic extract (250 mg/kg)	205.50 ± 2.39	198.00 ± 2.15*	191.50 ± 1.89*	182.50 ± 1.24*
F	Alloxan + Ethanolic extract (500 mg/kg)	206.50 ± 2.45	200.00 ± 2.07*	194.00 ± 1.69*	187.00 ± 1.50*

Values are Mean ± S.E.M; n=6

*P < 0.001 vs Diabetic Control.

Effect of *Ficus glomerata* leaf extract on biochemical parameters

Serum urea, serum creatinine and serum cholesterol levels were decreased significantly in a dose related fashion by ethanolic extract of *Ficus glomerata* (at 250 and 500 mg/kg) due to 10 days of treatment, where as serum protein level was increased significantly when compare to diabetic control group. However, the extract at dose of 100 mg/kg failed to reverse the altered biochemical parameters. Results are shown in Table No.2.

Effect of *Ficus glomerata* leaf extract on body weight in diabetic rat

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 10 days. Alloxan mediated body weight reduction was significantly reversed by the ethanolic extract in dose dependant fashion (at 250 and 500 mg/kg). The effect of test extract at 100 mg/kg on body weight of the animals was also found statistically not significant. Results are shown in Table No. 3

DISCUSSION

Pancreas is the primary organ involved in sensing the organism's dietary and energetic states via glucose concentration in the blood and in response to elevated blood glucose, insulin is secreted (2).

Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas (18, 19).

Alloxan causes a massive reduction in insulin release by the destruction of b-cells of the islets of langerhans, thereby inducing hyperglycaemia (20) Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases (21,22).

The results of the present study indicate that *Ficus glomerata* leaf extract was found to reduce the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present investigation ethanolic extract of *Ficus glomerata* leaf demonstrated the significant anti-diabetic activity. The results from the present study also indicate that *Ficus glomerata* leaf extract can reduce the levels of serum urea, serum creatinine, serum cholesterol and increase the serum protein and confirms the possibility that the major function of the extract are on the protection of vital tissues (Kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals.

The literature review indicated hepatoprotective property of *Ficus glomerata* leaf extract (23), the improvement of liver function and subsequent increase in uptake of blood glucose and its utilization may be another mechanism of action of the extract. Other possible mechanism includes the stimulation of β-cells and subsequent release of insulin and activation of the insulin receptors. Estimation of insulin level and insulin receptor may give more insight into the mechanism of the anti-diabetic activity exhibited by the extract.

The present study also indicates that *Ficus glomerata* can partially inhibit alloxan renal toxicity as observed from serum urea and creatinine levels.

The literature reports reveal that flavonoids and tannins present in the plant extract known to possesses antidiabetic activity. In the present investigation also the observed antidiabetic potential of test extract may be due to presence of similar phytoconstitutes which was evident by preliminary phytochemical screening.

CONCLUSION

From this study, we can state that the Ethanolic extract of Ficus glomerata has beneficial effects on blood glucose levels as well as improving hyperlipidemia and other metabolic aberrations. Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will be helpful in projecting this plant as an therapeutic target in diabetes research.

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