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Research Article

ANTI-DIABETIC ACTIVITY OF *FICUS NERVOSA* LEAF IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetes mellitus is the most common endocrine disorder that impairs glucose homeostasi resulting in severve diabetic complications. In the present study diabetes was induced i albino rats model with alloxan monohydrate. The effect of ethyl acetate and ethanoli extract of *Ficus nervosa* leaf was under taken to screen the hypoglycemic activity. Th results showed that the ethanolic extract dose of 250mg/kg b.wt has significar antihyperglycemic effect in experimental model of diabetes mellitus.

Key words: *Ficus nervosa*, Alloxan, Diabetes mellitus, Blood glucose.

INTRODUCTION

Diabetes is the world largest endocrine disease associated with increased morbidity and mortality rate[1] affecting at least 15 million people having complications which include hypertension, atherosclerosis and microcirculatory disorders[2]. India has today become the diabetic capital of the world with over 20 million diabetes and this number is set to increase to 57 million by 2025[3]. Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when its fatal complications are taken into the account[4].

Diabetes mellitus 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].

A wide number of traditional medicinal plants are still being used to treat diabetes mellitus. Several beneficial role such as correcting alterated carbohydrate metabolism, maintaining integrity and function of β-cells, insulin secreting activity, enhancing glucose up take and utilization and antioxidant properties present in traditional medicinal plant and their constituents offer exciting opportunity to develop them in to novel therapeutics[9]. In the present study we selected a plant namely *ficus nervosa (*moraceae*)*. It is an monoecious evergreen medium sized trees used traditionally for its curative property in treating diabetes, rheumatism and ulcer disorders.[10] From the literature survey, *ficus nervosa* roots have been reported to contain several secondary metabolites like flavonids, coumarin, flavones, steroids, triterpenoids which also possesses the antimycobacterial activities[11]. However, the chemical constituents and biological activities of *ficus nervosa* leaf have never been investigated, thus the present study was initiated to evaluate anti diabetic activity of various extract of leaf of *ficus nervosa*.

Experimental Section

Material and Methods[12]

The plant of *ficus nervosa* HEYNE ex ROTH collected from the chittoor district was authenticated by Dr. K. Madhava chetty, Ph.D., Department of botany, S.V University, Tirupathi. Voucher Specimen no-0603. The leaves of *ficus nervosa* were shade dried after collection of 15 days and was coarsely powdered. The powdered leaf was defatted with petroleum ether and then subjected to continuous hot extraction in soxhlet apparatus with ethyl acetate and ethanol. The extract was filtered through a cotton plug, followed by whatmann filter paper (no.1). The extract was evaporated under reduced pressure using rotovac evaporator at a low temperature (40-60^{o}c).

Phytochemical studies[13]

The various extract of *ficus nervosa* were subjected to preliminary phytochemicals and it revealed the presence of alkaloids, flavoinds, terpenoids, steroids ,tannins and carbohydrates.

Animals

Wistar Albino rats (150-200mg) of either sex were used in this investigation. They were maintained at standard housing condition and fed with commercial diet (Hindustan lever ltd., Bangalore) and provided with water *ad libitum* during the experiments. The Institutional Animal Ethical Committee permitted the study.

Acute toxicity studies

Acute toxicity study was performed for various extracts of *ficus nervosa* according to the acute toxic classic methods as per OECD guidelines [14]. The animals were kept fasting for overnight providing only water, after which the various extracts were administrated orally at the dose of 2000mg/kg was prepared by dissolving the extract in distilled water and the concentration was adjusted in such a way that it did not exceed 1ml/100g of the rat. The extract was then administrated orally (p.o) and animals were observed for behavioral changes, any toxicity and mortality up to 48 hrs.

Induction of diabetes

Diabetes mellitus was induced by single i.p injection of freshly prepared solution of alloxan monohydrate (120mg/kg b.wt) dissolved in physiological saline in overnight fasted wistar rats[15]. The diabetes was assessed in alloxan induced rats by determining the blood glucose concentration. The rats with blood glucose level above 250mg/dl were selected for the experimental study.

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 in sterile saline. after 5 days of alloxan injection, the hyperglycemic rats (glucose level>250 mg/dl)were separated and divided in to 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 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 4th, 7th and 10th day of the treatment. On the 10th day, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia for biochemical estimation.[16]

Grouping of animals

Rats are divided into 7 groups, each group containing 6 rats, the treatment schedules of animals belonging to different groups are shown below.

Group A

Served as normal control food and distilled water.

Group B

Served as diabetic control 2 % alloxan monohydrate, 120 mg/kg body wt. This was followed by a daily administration of distilled water (10 ml/kg body wt per day). This group served as counted for group D, E, F and G.

Group C
Alloxan + Glibenclamide (10 mg/kg, p.o.) served as standard.

Group D
Alloxan + Ethanol extract of *Ficus nervosa*,(100 mg/kg, p.o.)

Group E
Alloxan + Ethanol extract of *Ficus nervosa* (250 mg/kg, p.o.)

Group F
Alloxan + Ethyl acetate extract of *Ficus nervosa*, (100 mg/kg , p.o.)

Group G
Alloxan + Ethyl acetate extract of *Ficus nervosa,(* 250 mg/kg, p.o.)

Results

Effects of *ficus nervosa* leaf extract on fasting blood glucose level in diabetic rats

A marked rise in fasting blood glucose level observed in diabetic control compare to normal control rats (Table:1). Ethanolic and ethyl acetate extract of *ficus nervosa* (at 100 & 250 mg/kg) exhibited a dose dependent significant anti hyperglycemic activity on 4th, 7th and 10th day post treatment. The ethylacetate (100 &250mg) extract dose also caused reduction in blood glucose level but the results were found stastically insignificant. The antihyperglycemic effect of ethanolic extract (100mg/250mg/kg) was found less effective than the reference standard glibenclamide produced a significant reduction in blood glucose compare to diabetic control.

Table 1: Effect of *Ficus nervosa* leaf extracts on fasting blood glucose level in Alloxan induced diabetic rats

Group	Treatment	Fasting blood glucose level(mg/dl)			
		Basal value	4th day	7th day	10th day
A	Normal control	90.43±3.77	91.72±2.92	92.32±1.63	88.20±3.34
B	Diabetic control(Vehicle)	292.8±5.20	287.9±5.15	291.2±5.46	289.4±9.72
C	Alloxan+Glibenclamide (10mg/kg)	284.86±6.91	205.23±7.00***	183.1±6.22***	178.13±6.22***
D	Alloxan+Ethanolic extract (100mg/kg)	290.76±4.78	260.72±5.55	237.23±8.12*	210.92±7.77**
E	Alloxan +Ethanolic extract (250 mg/kg)	285.48±5.33	240.99±6.68*	210.95±9.99**	196.06±9.87***
F	Alloxan+Ethyl acetate extract (100mg/kg)	291.76±4.58	265.72±5.32	257.23±8.90	249.92±7.20**
G	Alloxan +Ethylacetate extract (250 mg/kg)	285.48±5.33	257.99±6.38	230.95±8.99*	223.06±9.70**

Values are Mean ± SEM; n=6; *P<0.05, **P<0.01 , ***P<0.0001 Vs Diabetic control.

Effect of *ficus nervosa* leaf extract on biochemical parameters

Serum urea, serum creatinine &serum cholesterol levels were decreased significantly in a dose related fashion by ethanolic extract of *ficus nervosa* at 100 &250mg/kg due to 10 days of treatment, whereas protein level was increased significantly when compare to diabetic control group. However the ethylacetate extract at dose (100mg/kg) failed to reverse the altered biochemical parameters. (Table: 2)

Table 2: Effect of *Ficus nervosa* leaf extracts 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	30.28±2.74	0.54±0.02	106.44±2.09	6.14±0.13
B	Diabetic control(Vehicle)	62.92±1.19	1.45±0.05	178.99±1.47	4.72±0.99
C	Alloxan+Glibenclamide (10mg/kg)	33.92±1.28***	0.68±0.03***	121.8±2.25***	6.11±0.09***
D	Alloxan+Ethanolic extract (100mg/kg)	51.37±1.49**	1.01±0.03***	155.02±6.14***	5.76±0.11***
E	Alloxan +Ethanolic extract (250 mg/kg)	43.08±1.62***	0.85±0.01***	144.9±2.76***	5.92±0.12***
F	Alloxan+Ethyl acetate extract (100mg/kg)	60.87±1.48**	1.34±0.03**	171.03±1.24**	4.67±0.12**
G	Alloxan +Ethyl acetate extract (250 mg/kg)	57.07±1.82**	1.15±0.02**	150±3.22**	5.24±0.10***

Values are Mean ± SEM; n=6; **P < 0.01, ***P<0.001 Vs Diabetic control.

Effect of *ficus nervosa* 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 drug treatment. Alloxan mediated body weight reduction was significantly reversed by ethanolic extract in dose dependent fasion (100mg &250mg/kg). The effect of ethyl acetate extract 100mg/kg on body weight of the animals was found stastically not significant when compared to the standard glibenclamide.

Table 3: Effect of *Ficus nervosa* leaf extracts on Body weight in alloxan induced diabetic rats

Group	Treatment	Body weight of the animal(g)			
		Basal value	4th day	7th day	10th day
A	Normal control	202.54±2.65	203.0±2.75	205.90±2.78	209.92±2.88
B	Diabetic control(Vehicle)	203.65±2.88	172.20±2.29	158.00±2.56	146.00±1.74
C	Alloxan+Glibenclamide (10mg/kg)	206.12±2.84	203.20±2.32*	197.20±2.32*	191.90±1.60*
D	Alloxan+Ethanolic extract (100mg/kg)	206.02±2.76	178.00±2.41*	162.00±2.34*	153.2±1.6*
E	Alloxan +Ethanolic extract (250 mg/kg)	205.40±2.32	198.20±2.22*	191.32±1.89*	183.50±1.22*
F	Alloxan+Ethyl acetate extract (100mg/kg)	206.10±2.83	179.99±2.32*	164.00±2.21*	158.30±1.90*
G	Alloxan +Ethyl acetate extract (250 mg/kg)	206.14±2.45	180.87±2.18*	166.89±2.54*	162.42±1.70*

Values are Mean ± SEM; n=6; *P<0.001 Vs Diabetic control.

DISCUSSION

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[17]. Alloxan causes a massive reduction in insulin release by the destruction of β cells of the islets of langerhans, there by inducing hyperglycaemia[18]. The results in present study indicate that *ficus nervosa* 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. In the present investigation ethanolic extract of *ficus nervosa* leaf demonstrated the significant anti diabetic activity. The results from the study also indicate that *ficus nervosa* 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

production of vital tissues including pancreas, thereby reducing the causation of diabetes in the experimental animals. Overall results showing the antidiabetic activity of *ficus nervosa* leaves, the activity may be due to presence of chemical constituents like flavonoids, terpenoids in leaves.

CONCLUSION

From this study, we can state that the ethanolic extract of *ficus nervosa* has beneficial effects on blood glucose level as well as improving hyperlipidemia and other metabolic aberrations. It has the potential to impart therapeutic effects in diabetes.

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