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EVALUATION OF ANTI-DIABETIC ACTIVITY OF FICUS BENGALENSIS LINN IN ALLOXAN INDUCED RATS

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ABSTRACT:
The present study was aimed to evaluate the anti-diabetic activity of isolated compounds from aerial parts of Ficus bengalensis in alloxan induced diabetic rats. Diabetic wistar albino rats were treated with standard drug Glibenclamide and prepared drug extract in 150 mg/kg. Hypoglycemic effect was evaluated in these rats and the efficacy of isolated compounds was administered in alloxan induced diabetic rats. At the end of study period blood glucose level were statistically analyzed based on the results. Isolated fractions produced a significant reduction in blood glucose level when compared with non-treated diabetic rats. So the present research work was confirmed that the isolated compounds possess hypoglycemic effect significantly.
Keywords: Ficus begalensis, antidiabetic, allaxon induced, Diabetes mellitus.

INTRODUCTION

Before the advent of insulin, diabetes was treated with plant medicines. The plant kingdom represents a largely unexplored reservoir of biologically active compounds not only as drugs, but also as unique templates that could serve as a starting point for synthetic analogs and an interesting tool that can be applied for a better understanding of biological processes. Folkloric uses are supported by a long history of human experience[1]. Numerous biologically active plants are discovered by evaluation of ethno-pharmacological data, and these plants may offer the local population immediately accessible therapeutic products.

In the present study, investigated the antidiabetic properties of ethanolic extracts and chloroform fraction of aerial parts of *Ficus bengalensis* Linn on normal and alloxan induced diabetic rats.

MATERIAL AND METHODS

Plant material

The selection of plant species for study was based on their traditional use for diabetes treatment, the information being gathered from published sources and traditional healers. The plants aerial parts of *Ficus bengalensis* Linn was selected for the present studies.

Plant was collected from the India nearby region of Pune during the months of August and September. Taxonomic and ethno medicinal identification of the collected plant done from Director, Botanical survey of India, Pune, Maharashtra. Fresh matured aerial parts *Ficus bengalensis* Linn was collected in bulk, initially rinsed thoroughly with distilled water, shade dried for 15 days. The shade dried materials were coarsely powder by a mechanical grinder and preserved in a nylon bag in a deep freezer, till further use.

Preparation of extracts

The plant materials (1 kg) were initially defatted with petroleum ether and then extracted with alcohol using a Soxhlet apparatus. The yield of the plant extracts ethanol (95%) measured about 20 g each after evaporating the solvent using water bath. The standard extracts obtained from *Ficus bengalensis Linn* were then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening **.**

Preparation of fractions of crude extract

Ethanolic extract then fractionated using Petroleum ether, Chloroform and water .The ethanolic extract , chloroform and aqueous fraction obtained from *Ficus bengalensis Linn* was then stored in a refrigerator at 4°C for further use for phytochemical investigation and pharmacological screening. Petroleum ether fraction was not used in the study because of very less yield.

Animals

Healthy adult Male albino wistar rats, weighing 150–200 g were used for the Screening methods.

Investigational model for induction of diabetes

Diabetes was induced by intra-peritoneal injection of Alloxan monohydrate (150 mg/kg b.w.) dissolved in the in normal saline. Blood was withdrawn (0.1 ml) from the tip of the tail of each rat under mild ether anaesthesia. The blood glucose level was checked before alloxanisation and after alloxanisation regularly in 24h intervals. Animals 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 72 h after alloxanisation.[2]

Preparation of Interventions

The measured quantity of extracts and fractions of *Ficus bengalensis Linn* and the standard drug glibenclamide (5 mg/kg) was suspended in 25% Tween-20 in distilled water. The solvent, test samples and standard drugs were administered by oral route based on dose and corresponding weight of the animals. For oral administration of test, standard as well as Solvent Feeding needle no 21 was used.

Maintenance of animals and Exposure Conditions

Earlier to the experiments, the selected animals were housed in acrylic cages in standard environmental conditions (temp: 20–25^{0}C; relative humidity: 45-55 % under 12 hr light/dark cycle), feed with standard rat feed for 1 week in order to adapt to the laboratory conditions and water *ad libitum.* They were fasted overnight (12 hr.) before experiments, but were allowed free access to water. Six animals were used for each group of study[3,4]. All the experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and as per the experimental protocols duly approved by the Institutional Animal Ethical Committee (CPCSEA/IAEC/INV/919/2019).

Blood glucose level determination

Fasting blood glucose concentration was determined using a Glucometer (Optium), based on the glucose oxidase method. Blood samples were collected from the tip of tail at the defined time patterns.

Hypoglycemic activity study of extracts on normoglycaemic animals (Single dose treated)

The hypoglycemic activity is important in the diagnosis of diabetes mellitus. It determines the ability of drug to decrease blood glucose level. This method permits for the effect of the drug to be tested in the animal with a whole pancreatic activity. The contrast may give some information regarding mechanism of action. The animals were fasted for 18 h, but were allowed free access to water before and throughout the duration of the 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 the GOD/POD method using a glucose estimation kit from M/s. Sigma Diagnostics (India) Pvt. Ltd., Baroda, India. The normal rats were then divided into six groups of six animals each. Group I served as solvent control and received only vehicle (2 ml/kg) through the oral route, Group II received glibenclamide (5 mg/kg) and served as reference control. Groups III to VI received the extracts of *Ficus bengalensis Linn* at a dose of 200 and 400 mg/kg, respectively, through the oral route. Blood glucose levels were examined after 1, 2, 4, 6, 8 and 10 h of administration of a single dose of the test and control samples[5, 6].

Effect of the extracts on blood glucose level in alloxan induce hyper glycemic rats (Acute and sub-acute models)

The antihyperglycemic / anti-diabetic evaluation has been carried out by following two methods:

- Acute model (Single dose treated alloxan induced hyperglycemic rats)
- Sub acute model (Multi dose, 11 days treated alloxan induced hyperglycemic rats)

Acute model (In single dose treated alloxan induced hyperglycemic rats)

The acclimatized animals were kept fasting for 24 h with water *ad libitum* and injected intraperitoneally a dose of 150 mg/kg of alloxan monohydrate in normal saline. After 1 h, the animals were provided feed *ad libitum*. The blood glucose level was checked before alloxanisation and 24 h after alloxanisation as above. Animals 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 72 h after alloxanisation. The animals were segregated into seven groups of six rats in each. Group I served as normal control, Group II served as diabetic control and received vehicle (2 ml/kg) through the oral route. Group III received glibenclamide (5 mg/kg). Groups IV to VI received extracts of *Ficus bengalensis Linn* at doses of 150mg/kg. Blood glucose level of each rat was estimated at 1, 2, 4, 6, 8 and 10 h, respectively.

Sub-acute model (In multi dose treated alloxan induced hyperglycemic rats)

The animals were kept fasting for 24 h with water *ad libitum* and injected alloxan monohydrate intraperitoneally at a dose of 150 mg/kg in normal saline. After 1 h, the animals were provided rat diet *ad libitum*. The blood glucose level was measured 72 h

after administration of alloxan. The animals showing blood glucose level beyond 200 mg/dl were considered for the study. The diabetic animals were segregated into seven groups of six rats each. Group I served as normal control, Group II served as diabetic control and received only vehicle (2 ml/kg) through the oral route. Group III received glibenclamide (5 mg/kg); Groups IV to VI received extracts of *Ficus bengalensis Linn* at doses of 150 mg/kg for 11days. The Group I served as normal reference. The blood glucose level was measured on 0, 3, 7 and 11th day of treatment.

RESULTS

Acute oral toxicity studies of ethanolic extract and its fractions of *Ficus bengalensis Linn*

Acute toxicity studies conducted revealed that the administration of graded doses of etanolic extract and both the fractions (up to a dose of 3000 mg/kg) of *Ficus bengalensis Linn* did not produce significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and appearance of the animals. No death was observed up to the dose of 3 g/kg body weight. The mice were physically active. These effects were observed during the experimental period (72 hrs). The result showed that in single dose; the plant ethanolic extract and fractions had no adverse effect, indicating that the medium lethal dose (LD_{50}) could be greater than 3 g/kg body weight in mice. Based on these results 1/5th of the maximum safest dose was taken for further pharmacological screening. So the doses selected for further study were 150 mg/kg b.w. for extract and test fractions.

Effects of Ethanolic extract and it's fractions of *Ficus bengalensis Linn* on single dose treated normoglycemic animals (hypoglycemic activity)

The effect of the ethanolic extract and fractions on blood glucose level (BGL) in normoglycemic rats is depicted in table1. The ethanolic extract at 150mg/kg dose level registered 77.69 mg/dl and 79.87 mg/dl BGL at the end of 10 hrs of the study, while it was 74.87 and 76.82 mg/dl with dose level of 200 and 400mg/kg dose for chloroform fraction. However, at the same time the standard drug glibenclamide at 5mg/kg showed 71.63 mg/dl of BGL. However the calculated percentage fall of BGL demonstrated 16.83, 17.88 and 16.79% with respect to 150 mg/kg dose level when measured at the end of the 10 hrs of the study, while at the same time glibenclamide showed 21.65% fall of BGL. The progressive fall of BGL of the test extract and fractions, in different test hour showed a statistical significant of $p < 0.05$ to $p < 0.01$, while analyzed by using ANOVA followed by Dunnet's t-test. The chloroform fraction possesses more BG lowering potency than that of the ethanolic extract. The test extract and both fractions at tested dose level also showed a significant fall of BGL while compared with the solvent control group during the study period of 1, 2, 4, 6, 8 and 10 hrs.

Effects of ethanolic extract and its fractions of *Ficus bengalensis Linn* on FBG levels on alloxan induced diabetic rats (21-days Sub acute model)

The study reveals that daily oral administration of ethanolic extract, and fractions reduces the blood glucose level to an extent of 123.34, 120.20 and 138.0 mg/dl at 200 and 400 mg/kg dose, respectively at the end of 21-day of the study, while at the same day standard drug glibenclamide at 5 mg/kg showed 95.33 mg/dl BGL. Moreover the calculated percentage fall of BGL demonstrated 56.52, 57.24 and 52.63% when measured at the 21-day of the study, while at the same time glibenclamide showed a 61.87% fall of BGL. The progressive fall of BGL of the test extract/fractions, in different test days showed a statistical significant of $p < 0.05$ to $p < 0.01$, while analyzed by using ANOVA followed by Dunnett's t-test. In the study chloroform fraction have more BG lowering potency than that of ethanolic extract. The results of the alloxan induced diabetes model showed that the ethanolic extract and test fractions of *Ficus bengalensis Linn* have antidiabetic effect. The results are summarized in table 2

Table 1: Effect of ethanolic extract and its fractions of *Ficus bengalensis Linn* on normoglycemic rats.

Groups	Treatment and Dose	Blood glucose level (mg/dl)							
		0	1	2	4	6	8	10	% decrease at 10^{th} hr
I	Diabetic control (tween+ water)	96.5 ± 4.80	95.66 ± 4.66	94.5 ± 4.77	99.16 ± 4.04	98.83 ± 4.15	99.83 ± 5.60	101.16 ± 4.90	-
II	Glibenclamide (5mg/kg)	91.43 ± 1.31	81.22 ± 2.63	67.53 ± 2.34*	58.12 ± 2.61**	54.72 ± 2.44**	73.83 ± 1.42**	71.63 ± 2.81**	21.65
III	Ethanolic extract (150mg/kg)	93.42 ± 1.63	88.33 ± 2.82	87.21 ± 1.24*	86.75 ± 1.42**	84.21 ± 1.89**	83.55 ± 1.97**	77.69 ± 2.07**	16.83
IV	Chloroform fraction (150mg/kg)	91.18 ± 0.93	87.19 ± 0.78	85.71 ± 2.61	84.23 ± 1.37	82.83 ± 2.38*	80.11 ± 1.21**	74.87 ± 2.73**	17.88
V	Aqueous fraction (150 mg/kg)	92.34 ± 1.23	89.23 ± 2.32	88.01 ± 1.21*	87.76 ± 1.42**	85.21 ± 1.89**	83.65 ± 1.67**	76.83 ± 2.07**	16.79

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to group-I).

Table 2: Effect of ethanolic extract and its fractions of *Ficus bengalensis Linn* on blood glucose level in alloxan induced diabetic rats (Sub acute model)

Groups	Treatment and Dose	Blood glucose level (mg/dl)					
		0	5	10	15	21	% decrease at 21st day
I	Solvent control (tween+ water)	82.40 ± 1.63	83.04 ± 2.30	82.20 ± 1.96	81.00 ± 1.30	81.00 ± 1.39	--
II	Diabetic control	264.0 ± 6.48	292.22 ± 7.73	308.00 ± 6.11	314.20 ± 3.32	325.20 ± 3.51	--
III	Glibenclamide (5mg/kg)	250.23± 7.50	203.24± 7.43**	149.00± 6.56**	129.02± 7.22**	95.33 ± 5.44**	61.87%
IV	Ethanolic extract (150mg/kg)	283.73± 4.13	231.12 ± 8.21*	203.73 ± 7.24*	186.78± 4.58**	123.34± 5.47**	56.52%
V	Chloroform fraction (150mg/kg)	281.11± 3.60	230.10 ± 9.21*	205.33 ± 7.24*	188.00± 5.68**	120.20± 6.65**	57.24%
VI	Aqueous Fraction (150 mg/kg)	291.33± 4.40	242.33 ± 5.36*	212.00± 4.35**	188.66± 4.40**	138.00± 4.97**	52.63%

Values are expressed in MEAN ± S.E.M of six animals. One Way ANOVA followed by Dunnet's t-test (t-value denotes statistical significance at *p<0.05, **p<0.01 respectively, in comparison to diabetic control group).

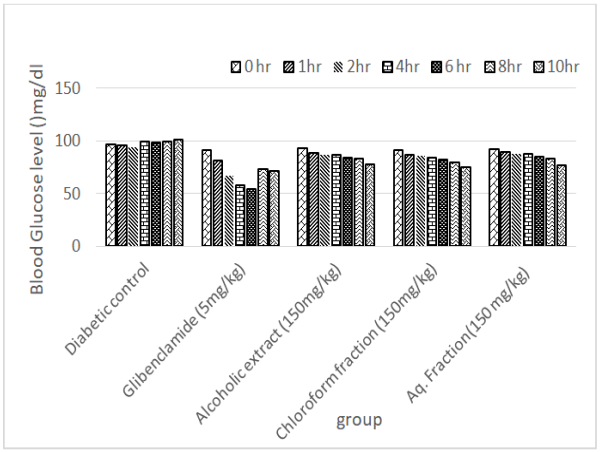

Figure 1: Effect of ethanolic extract and its fractions of *Ficus bengalensis Linn* on normoglycemic rats

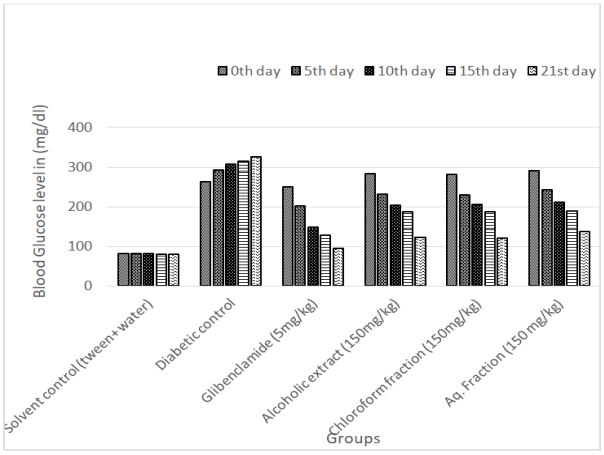

Figure 2: Effect of ethanolic extract and its fractions of *Ficus bengalensis Linn* on blood glucose level

CONCLUSION

Effect of ethanolic extract of *Ficus bengalensis Linn* on oral glucose tolerance in normal rats & alloxan induce hyper glycemic rats (Acute model)

The ethanolic extract of aerial parts of *Ficus bengalensis Linn* produced antidiabetic effect in single dose treated animals. The test result indicates that, there is a significant reduction (p<0.01) in blood glucose level from 2h (p<0.05) onwards till the end of 10h and registered 57.39%, and 71.04 % reduction at the end of 10h, in animals treated with 200 and 400mg/kg of the extract. However the standard drug glibenclamide at the same time reduces the blood glucose 71.26% with p<0.01, when compared with solvent control group. The results showed that the ethanolic extract of *Ficus bengalensis Linn* have significant antidiabetic effect.

The oral glucose tolerance test in the present investigation demonstrated that ethanolic extract , at the tested dose levels, are having significant control over the blood glucose levels in the glucose loaded hyperglycemic animals.

Effect of ethanolic extract and its fractions on fasting BGL in normal and diabetic rats

The results of the investigations revealed that treatment with ethanolc extract and fractions produced hypoglycemia in normoglycemic (euglycemic) rats. The test result indicates that, there is a significant reduction (p<0.05) in blood glucose level from 2h (p<0.05) onwards till the end of 10h and registered 16.83%, 17.88% and 16.79 % reduction at the end of 10h, in animals treated with 150mg/kg of the ethanolic extract and chloroform fractions of *Ficus bengalensis Linn*. However the standard drug glibenclamide at the same day reduces the blood glucose 21.65% with p<0.01, when compared with solvent control group. The results of the normoglycemic model showed that the test fractions and ethanolic extract have hypoglycemic effect.

While ethanolic extract and test fractions of *Ficus bengalensis Linn* reduces the blood glucose level to an extent of 56.52%, 57.24% and 52.63% at 150 mg/kg body weight respectively at the end of the 21st day of the study, where as the standard drug glibenclamide registered 61.87% of reduction at the same day of the study. Though chloroform fraction more significantly reduce blood glucose level as compared to ethanolic extract in diabetic rats. However the individual data shows a statistical significance ranges between p<0.05 to p<0.01, throughout the experiment when compared with solvent control and analysis of variance registered p<0.01 level of significance. Treatment with ethanolic

extract, and chloroform fraction of *Ficus bengalensis Linn* in alloxan -induced diabetic rats started reducing fasting blood glucose levels 5 days and made them normoglycemic after 21 days.

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