

# Evaluation of the phytochemicals and antidiabetic activity of *Ficus bengalensis*

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*Ficus bengalensis* Linn, commonly known as the banyan tree, belongs to the Moraceae family. Its bark is used for the treatment of diabetes. In the present study, the ethanolic extracts of the different aerial parts of *Ficus bengalensis* Linn were comparatively evaluated for their blood glucose lowering activity. Histopathology of the treated groups was carried out to evaluate the betacytotropic activity of various parts of *Ficus bengalensis*. The ethanolic extract of the fruit, at a dosage of 120 mg/kg body weight, was found to exert a more pronounced antidiabetic activity than the ethanolic extract of the root or bark. The experiment also confirmed the antidiabetic activity of the standard drug glibenclamide.

**KEY WORDS:** Alloxan, antidiabetic activity, bark, *Ficus bengalensis*, fruit

Diabetes is the most common disease associated with carbohydrate metabolism and is a major cause of disability and hospitalization.<sup>[1]</sup> Type 2 diabetes is by far the commonest form of the disease globally, with rapidly developing countries being at the forefront of this epidemic. Current estimates are that at least 150 million people worldwide have diabetes, of which two-thirds live in developing countries. The total number of people with diabetes is predicted to rise to about 300 million by 2025, with one-third of affected individuals living in India and China alone.<sup>[2]</sup>

The use of ethnobotanicals has a long folkloric history for the treatment of blood sugar abnormalities. The World Health Organization has estimated that 80% of the world's population use botanical medicine for

their primary healthcare needs. The *Ficus bengalensis* Linn, commonly known as the banyan tree, is member of Moraceae family and its bark is used in Ayurvedic medicine for the treatment of diabetes mellitus.<sup>[3,4]</sup> The water and alcoholic extract of bark of this plant have been shown to produce a hypoglycemic effect in experimental models following oral administration.<sup>[5-7]</sup> Some investigators have attempted to purify the active fractions from the bark of *Ficus bengalensis* in order to establish the mechanism of its hypoglycemic action.<sup>[5]</sup> All the studies have been restricted to its bark, so the other morphological parts, i.e., the fruits, leaves and aerial roots deserve evaluation for their antidiabetic activity. In the present investigation, extract of the fruit and aerial root were evaluated for antidiabetic activity and compared with the extract of bark for their efficacy in lowering blood glucose.

## Methodology

Aerial roots, fruits and bark of *Ficus bengalensis* (FB) were collected from the Agriculture College of Mandsaur and positively identified and certified by a botanist in the KNK College of Horticulture, Mandsaur Agricultural College. Samples of all morphological parts (voucher specification no. B/2006) were deposited in the herbarium of the Department of Pharmacognosy, B.R. Nahata College of Pharmacy, Mandsaur (M.P.). The materials were cleaned thoroughly with distilled water to remove any type of contamination. Washed bark and fruits were air dried in shade, while the aerial roots were dried in an electric oven at a temperature 55°C for 4 h.

## Preparation of alcoholic extract

The plant parts (fruits, aerial roots and bark) were separately powdered in a grinder and 20 gm of each powder was filled in the Soxhlet apparatus for extraction. The whole assembly of the Soxhlet apparatus was set up and first defatted by petroleum ether (60-80°C) for

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72 h. After complete defatting, the drug powders were dried in room temperatures and extracted with ethanol for 48 h. The alcoholic extracts of the different parts were concentrated and stored in airtight containers until needed for further studies.

The extracts were concentrated under reduced pressure at a low temperature (40–50°C). The alcohol-extractive values of the different morphological parts, i.e., fruits, aerial roots and bark of *Ficus bengalensis* were 5.8, 6.2, and 6.6%, respectively.

Male albino rats (130-185 g) were procured from the department's animal house and were acclimatized for at least 7 days prior to commencement of experiment. The rats were randomly distributed to different groups and placed in plastic cages. Each group was composed of six animals; the mice were numbered. Experimental animals were maintained on standard pelleted laboratory animal feed and water *ad libitum*. Animals were maintained at 22 ± 2°C and 55 ± 5% relative humidity in a light-controlled (14 h light/10 h dark) room. Animals that are described as fasting were deprived of food for at least 16 h but were allowed free access to drinking water. The Institutional Animal Ethics Committee (IAEC) approved all the experiment protocols.

### Induction of diabetes

All the rats were fasted overnight before the administration of alloxan. Alloxan was given by intravenous route through the tail vein in three equally divided dose. The total dose was 180 mg/kg of alloxan. The alloxan was dissolved in 0.9% w/v saline solution. Freshly prepared solution was administered. The first dose was given on day zero; the second dose on the third day, and the third dose on the fifth day. Control rats were injected with saline only.

After a fortnight, urine sugar estimation was done by Benedict's method. The rats with high urine sugar and >200mg/dl plasma glucose were selected for the diabetic group and were stabilized for 30 days before starting the extract treatment.<sup>[8]</sup> Alcoholic extract of the drug was suspended in 2% acacia solution and the dose was given, between 10.00 am to 12.00 am, by the oral route using a catheter. Each dose contained 120 mg/kg extract. In the case of glibenclamide, each dose contained 0.5 mg/kg of the drug.

### Experimental design

In the experiment the rats was divided into the following

groups, with six animals in each:

Group A: control; fed 5 ml of tap water daily

Group B: Diabetic control; fed 5 ml of tap water daily

Group C: Diabetic rats; treated with ethanolic extract of fruits (120 mg/kg PO) daily

Group D: Diabetic rats; treated with ethanolic extract of aerial roots (120 mg/kg PO) daily

Group E: Diabetic rats; treated with ethanolic extract of bark (120 mg/kg PO) daily

Group F: Diabetic rats; treated with glibenclamide. (0.5 mg/kg PO) daily

Fasting blood samples were collected from the orbital sinus of the eye at different time intervals and estimated by GOD-POD kit method. The decreases in percentage of glucose level in experimental animal were calculated using by formula given below:

$$\frac{\text{Before treatment} - \text{After treatment}}{\text{Before treatment}} \times 100$$

Data are expressed as mean ± SEM and was analyzed for significance by Dunnet's test (comparing all *vs* control) using InStat v.2.02 software (GraphPad Software Inc.). *P* value <0.001 was considered significant.

### Histopathological studies

At the end of the study, 50 rats were sacrificed and the pancreas collected. The tissue was fixed in 10% formalin immediately after removal from the animal to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol dehydration and infiltration of xylene or chloroform as a solvent for wax.

Thin sections of the tissue (7 μm) were cut using a microtome and these were stained with hematoxylin-eosin. The tissue sections were subjected to rehydration by exposure them to decreasing concentrations of alcohol from 100–30% and then stained with hemotoxylin, which has an aqueous base. The sections were dehydrated using increasing concentrations of alcohol and then stained with eosin. They were then treated with diphenyl xylene (DPX) and examined under the microscope.<sup>[9]</sup>

### Results

In all groups, prior to alloxan administration, the basal blood glucose levels of the rats were not significantly

different. However after alloxan administration blood glucose levels were significantly higher and these animals were selected for the study group. The nondiabetic control rats remained consistently euglycemic throughout the course of the study.

Thirty-six rats were taken and marked separately, viz, control rats, untreated rats, and rats treated with ethanolic extract of one of the following: aerial roots, fruits, bark, or the standard drug, glibenclamide. The experiment was carried out for 50 days. All the rats were fasted overnight before the administration of alloxan. Alloxan was given by intravenously through the tail vein in three divided doses for the induction of diabetes. The investigation was carried out by administration of the ethanolic extracts and standard drug after 30 days when the diabetes was stabilized. Control rats were injected with saline only.

After 30 days, the rats with high blood sugar of >200 mg/dl plasma glucose were selected for the experiment. Treatment was carried out with ethanolic extract of aerial roots, fruits, or bark as well as the standard drug, glibenclamide. 120 mg of the drug was given in 5 ml of water once daily by the oral route. The alcoholic extracts of aerial root, fruits and bark and the standard drug glibenclamide brought about 18.33, 31.72, 28.84 and 34.43% reduction in blood glucose levels, respectively; ( $P < 0.01$ ) [Table 1].

### Histopathological studies

Stained pancreatic section showed that alloxan caused severe necrotic changes in the pancreatic islets, especially at the center of the islets. This consisted of nuclear changes, disappearance of the nucleus and, in some places, relative reduction in the size and number of islets

with a residue of destroyed cells; this was seen especially around the large vessels. There was also severe reduction of the number of  $\beta$  cells. Study of the pancreas in the treated groups did not show any significant differences as compared with the diabetic control group.

### Discussion

In the present work, we investigated the comparative hypoglycemic effects of the ethanolic extracts of different parts of *Ficus bengalensis* in the alloxan diabetic model. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas.<sup>[10,11]</sup> *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, causing cell necrosis.<sup>[12]</sup> The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells.<sup>[13]</sup>

There is a wide variability in the dose of alloxan required to produce a long-lasting diabetic state that is also compatible with life. Different doses of alloxan produce varying intensities of hyperglycaemia ranging from 160 to 400 mg/dl.<sup>[14-16]</sup>

We showed that oral administration of ethanolic extracts of the fruit, aerial root and bark of *Ficus bengalensis* for 21 days produced significant decrease in blood glucose, i.e., decreases of 31.73, 18.33, and 28.84%, respectively. Oral administration of glibenclamide (0.5 mg/kg P.O.) showed the maximum reduction (34.4%) in blood glucose level [Table 1].

The present study clearly reveals that the ethanolic extract of fruits produces the maximum reduction in

**Table 1: *In vivo* evaluation of different extracts of *Ficus bengalensis* Linn. for antidiabetic activity**

Groups ( <i>n</i> =6 in each group)	Blood glucose level (mean $\pm$ SD) (mg/dl)					Percent lowering of blood glucose (day 0 vs day 15)
	Basal	0 <sup>th</sup> day	5 <sup>th</sup> day	10 <sup>th</sup> day	15 <sup>th</sup> day	
Normal control	274.0 $\pm$ 3.62	120 $\pm$ 0.856	119 $\pm$ 0.966	119 $\pm$ 1.0966	120 $\pm$ 1.862	
Diabetic control	73.65 $\pm$ 3.22	289 $\pm$ 1.714	288 $\pm$ 1.605	290 $\pm$ 1.732	287 $\pm$ 0.640	
Ethanolic extract of Aerial root (120 mg/kg body wt.)	75.21 $\pm$ 3.42	289 $\pm$ 1.414	268 $\pm$ 2.472	252 $\pm$ 1.414	236 $\pm$ 4.201 18.33	$P < 0.01$
Ethanolic extract of fruit (120 mg/kg body wt.)	74.52 $\pm$ 3.42	290 $\pm$ 1.732	246 $\pm$ 1.321	230 $\pm$ 1.321	198 $\pm$ 6.616 31.72	$P < 0.01$
Ethanolic extract of bark (120 mg/kg body wt.)		260 $\pm$ 1.32	229 $\pm$ 1.313	203 $\pm$ 1.321	185 $\pm$ 5.956 28.84	$P < 0.01$
Glibenclamide (0.5 mg/kg body wt.)	75.5 $\pm$ 6.4	302 $\pm$ 0.579	270 $\pm$ 1.533	242 $\pm$ 0.579	198 $\pm$ 7.975 34.43	$P < 0.01$

blood glucose level as compared to the extract of aerial root or bark of *Ficus bengalensis*. The ethanolic extracts of various parts of *Ficus bengalensis* were tested on laboratory animals and histopathology was carried out on each treated group to assess the betacytotropic activity of the different extracts. Histopathological studies of untreated diabetic rats showed almost complete destruction of beta cells due to the alloxan. Diabetic rats which were treated with ethanolic extract of fruits showed almost normal cells. It seems that the extract either protected the cells from the toxic effect of alloxan or the cells recovered after the initial injury. In the treated group, cytoplasmic granulation in the beta cells is visible, though not as much as in the normal case.

We concluded that ethanolic extract of fruits of the *Ficus bengalensis* is more effective than the extract of other morphological parts of the same plant. The alcoholic extracts of aerial roots and bark also brought down the blood glucose level, though not as much as the extract of fruit. The experiment also revealed the antidiabetic activity of standard drug, glibenclamide.

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