

Antidiabetic activity of fruit pulp of *Feronia elephantum* Corr.

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ABSTRACT

Feronia elephantum Corr. Commonly known as Bela, Billin, Kath, is a commonly known herb in Indian system of medicine to treat various disorders including diabetes mellitus without any scientific evidences. Therefore this study was designed to investigate *in vivo* hypoglycemic and antidiabetic potential of methanolic extract of fruit pulp of *Feronia elephantum* Corr. in glucose loaded animals and alloxan induced diabetic animals. In both the models *Feronia elephantum* Corr. reduce the blood glucose level when compared to diabetic control group and exert a significant hypoglycemic and antidiabetic activity. However the potency of the herb was less than that of standard drug metformin. *Feronia elephantum* Corr. methanolic extract also reduced the rate of body weight loss in normal and alloxan induced diabetic animals. The results of this study revealed the presence of a significant antidiabetic potential of methanolic extract of *Feronia elephantum* Corr. in alloxan induced diabetic rats. On the basis of this further research work is needed to investigate exact mechanism of action and also to isolate the active constituent/s responsible for the activity.

Key words: Diabetes mellitus, Glucose, Metformin, *Feronia*, Alloxan

INTRODUCTION

Diabetes mellitus (DM) currently is a major health problem for the people of the world and is a chronic metabolic disorder resulting from a variable interaction of hereditary and environmental factors and is characterized by abnormal insulin secretion or insulin recaptor or post receptor events affecting metabolism involving carbohydrates, proteins and fats in addition to damaging liver, kidney and β cells of pancreas.^[1] The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million peoples likely to be diabetic by the year 2030 as against 191 million estimated in 2000.^[2] From literature review it has been revealed that 15 - 20% of diabetic patients are suffering from insulin-dependent diabetes mellitus (IDDM) or type-I.^[3] In Type-I diabetes mellitus, there is completely destruction of pancreatic β cells and patient is unable to release insulin for maintaining the blood glucose.

In Type-II diabetes mellitus pancreatic β cells partially destruct and/or formation of such proteins opposing the insulin action. The IDDM is noted both in adult and child hood. It is characterized by elevation of both fasting and post-prandial blood sugar levels. Chronic hyperglycemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries.^[4] These may be delayed, lessened or prevented by maintaining blood glucose values close to normal in modern medicine; no satisfactory effective therapy is still available to cure the diabetes mellitus. Though insulin therapy is also used for the management of diabetes mellitus, but there are several drawbacks like insulin resistance^[5], anorexia, nausea, brain atrophy and fatty liver after chronic treatment. Besides the use of insulin for the treatment of insulin dependent diabetes mellitus (IDDM), other approaches for the control of hyperglycemia include the use of amylin analogues which regulate gastric emptying and inhibitors of intestinal alpha glucosidases like acarbose, miglitol and voglibiose which delay postprandial hyperglycemia. Sulphonylureas, the most widely used class of drugs act by closure of ATP dependent channel. Metformin, a biguanide oral antidiabetic limits intestinal glucose absorption. These drugs have certain effects like causing hypoglycemia at higher doses, liver problems, lactic acidosis and diarrhea. It is apparent that due to the side effects of the currently used drugs, there is a need for a

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safe agent with minimal adverse effects, which can be taken for long durations. Though biguanides and sulfonylureas are valuable in treatment of diabetes mellitus, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects.^[6] Moreover, these therapies only partially compensate for metabolic derangements seen in diabetics and do not necessarily correct the fundamental biochemical lesion.^[7]

Recently, there has been increasing interest in the use of medicinal plants. The use of medicinal plants in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases. However today it is necessary to provide scientific proof as whether to justify the use of plant or its active principles.^[8]

Feronia elephantum (Corr.) (common names: Bela, Billin, Kath, Kavitha), belongs to family Rutaceae, is a native of Indian subcontinent. The fruit pulp of the plant has been reported in traditional medicine as a curative for various ailments such as diarrhoea, pruritis, impotence, dysentery, heart disease, vomiting, and anorexia, and has also been used for the treatment of asthma and tumours, and as a liver tonic.^[9] A decoction (Kadha) administered orally before breakfast has been advocated by local traditional medical practitioners as a tonic purpose.^[10] The fruit pulp of *Feronia elephantum* (Corr.) contains flavonoids, phytosterols, tannins, carbohydrates, triterpenoids and amino acids as its chemical constituents.^[11]

The gum of the plant are widely used as a curative for diabetes mellitus in Indian system of medicine and also used as a folklore remedy to control the blood glucose level.^[10] Hence this study was undertaken to investigate the effect of methanolic extract of *Feronia elephantum* Corr. fruits pulp in normal and alloxanised diabetic rats.

MATERIALS AND METHODS

Plant material

The fruit pulps of *Feronia elephantum* Corr. were collected from the local market of Varanasi and were authenticated by Division of taxonomy, National Botanical Research Institute (NBRI), Lucknow and a voucher specimen no. NBRI/CIF/108/2010 was deposited in national herbarium of NBRI, Lucknow for future reference.

Extract preparation

The dried fruit pulp of the plant (250 g) was comminuted to powder passing through a 60 mesh and then extracted with 95 % methanol using a Soxhlet apparatus. The extract was filtered through cotton wool plug and dried in vacuum

on a rotary evaporator (buchi type) at 40-50°C. Complete dryness was achieved in a calcium chloride desiccator and the dry extract was used for all experimental studies.

Phytochemical screening

The preliminary phytochemical screening of the crude methanolic extract of fruit pulp of *Feronia elephantum* Corr. was carried out in order to ascertain the presence of its constituents utilizing standard conventional protocols.^[12-14] Phytochemical screening using thin layer chromatography (TLC) was conducted for extract that showed a potential antidiabetic activity against alloxan induced rat diabetes model. Methnolic extract was applied 1 cm above from the base of the TLC plates (0.25 mm, Macherey-Nagel, Germany). Developmet was done using Toluene: methanol: diethyl amine (60:40:5) and Chloroform: methanol: Glacial Acetic Acid (9:1:1) as solvent system specific for various flavonoids and terpenoids respectively. Development of the chromatograms were carried out using anisaldehyde-sulphuric acid spraying reagent and then plates were heated at 105°C in a hot air oven till the spots were developed.^[15]

Animals

Healthy male albino Wister rats each weighing 150-200 g were used for study. The rats were housed in polypropylene cages in animal house of BBDNITM, Lucknow and maintained under standard conditions (12 h light and dark cycles, 25 ± 3°C and 55-60% relative humidity). The animals were fed with a standard diet and water *ad libitum*. The study was performed as per the guidelines of IAEC (Institutional Animal Ethics Committee) and Committee for purpose of control and supervision of Experiments on Animals (CPCSEA) and was approved by approval no. BBDNITM/IAEC/01/2010.

Acute toxicity study

The 'Up and Down' or 'Staircase' method was adopted for this evaluation. The extract was administered orally in a dose range of 200-5000 mg/kg body weight to ten groups of mice ($n = 6$). Two mice were orally dosed with 250 mg/kg and observed for a period of 24 h for mortality. In this approach, subsequent doses were then increased by a factor 1.5 if the dose was tolerated, or, decreased by a factor of 0.7 if it was lethal. The maximum non-lethal and minimum lethal doses were determined using 6 mice. Those mice which received doses above 5000 mg/kg body weight exhibited ptosis (drooping of upper eyelid) and were observed to be lethargic. Once the approximate LD₅₀, or the range between the maximum non-lethal and minimum lethal dose was found, a final and more reliable LD₅₀ assay was performed using at least 3 or 4 dose levels within this range with a larger number of animals in each group. In addition, the source of animal, sex, age, body weight, and presence or absences of any immediate reaction were also recorded as per CPCSEA protocol.^[16] The duration of the toxicity test was two weeks.

Induction of diabetes mellitus

Twenty four male Albino Wistar rats weighing 150-200 grams were used for the study of the effects of *Feronia elephantum* extract on the blood glucose levels of the animals. The animals were fed on commercial feeds and were given water *ad libitum*. The animals were fasted from feeds for 12 hours before the commencement of each experiment, but were allowed water *ad libitum*. The rats were injected with alloxan mono hydrate suspended in water at a dose of 120 mg/kg body weight intraperitoneally. They were kept for the next 24 hours on 5% glucose solution bottles in their cages to prevent hypoglycemia. After a period of three days the rats with a blood glucose levels greater than 150 mg/dl were considered diabetic and used for current research work.^[17]

Experimental procedure for ogtt and antidiabetic activity

The animals for oral glucose tolerance test were randomly assigned into four groups of six rats in each group ($n = 6$) each as follows, namely

Group 1 - Normal undiseased animals who only received normal saline (Normal Control).

Group 2 - Diseased animals who received glucose (1.75 g/kg p. o.)

Group 3 - Diseased animals who first received metformin 100 mg/kg and then glucose (2 hrs. later) 1.75 g/kg p. o.

Group 4 - Diseased animals treated methanol extract of *Feronia elephantum* Corr. 500 mg/kg

Oral glucose tolerance tests were performed on 16 hr fasted Wister rats using 1.75 g glucose per kg body weight fed orally (dissolved in water for injection) through a canula fitted needle attached to syringe. Just after glucose fed single dose of plant sample (methanolic extract of fruit pulp of *Feronia elephantum* Corr.) was also fed to study the effect of the same on GTT. The control group were fed equal amount of vehicle solutions orally.^[17]

In all the groups, blood was collected from the animal's tail vein at 0, 30, 60, 90 minutes after glucose feeding. Simultaneously, blood glucose was measured by trinder's glucose oxidase method using spectrophotometer.

Table 1: Effect of extract on oral glucose tolerance test in normal rats

Groups	Dose	Blood glucose level (mg/dl) (mean \pm sem)			
		0 minute	30 min	60 min	90 min
Group I (normal control)	Normal saline	103.83 \pm 2.49	105.65 \pm 2.94	104 \pm 3.36	104.59 \pm 2.61
Group II (disease control)	1.75 g/kg	105.05 \pm 1.54	201.66 \pm 2.40	267.83 \pm 1.60	198.67 \pm 1.74
Group III (standard)	100 mg/kg	104.30 \pm 1.52**	115.95 \pm 1.50**	115.70 \pm 3.99**	101.5 \pm 1.23**
Group IV (Test)	500 mg/kg	107.38 \pm 1.91**	130.00 \pm 2.51**	134.46 \pm 2.74**	123.0 \pm 2.08**

Group I - normal control; Group II - Diseased control; Group III - Diseased animals treated with standard drug Metformin; Group IV - Diseased animals treated methanol extract of *Feronia elephantum* Corr. 500 mg/kg

Values are expressed in Mean \pm SEM ($n = 6$), $P^{**} < 0.05$ when compared to Group II.

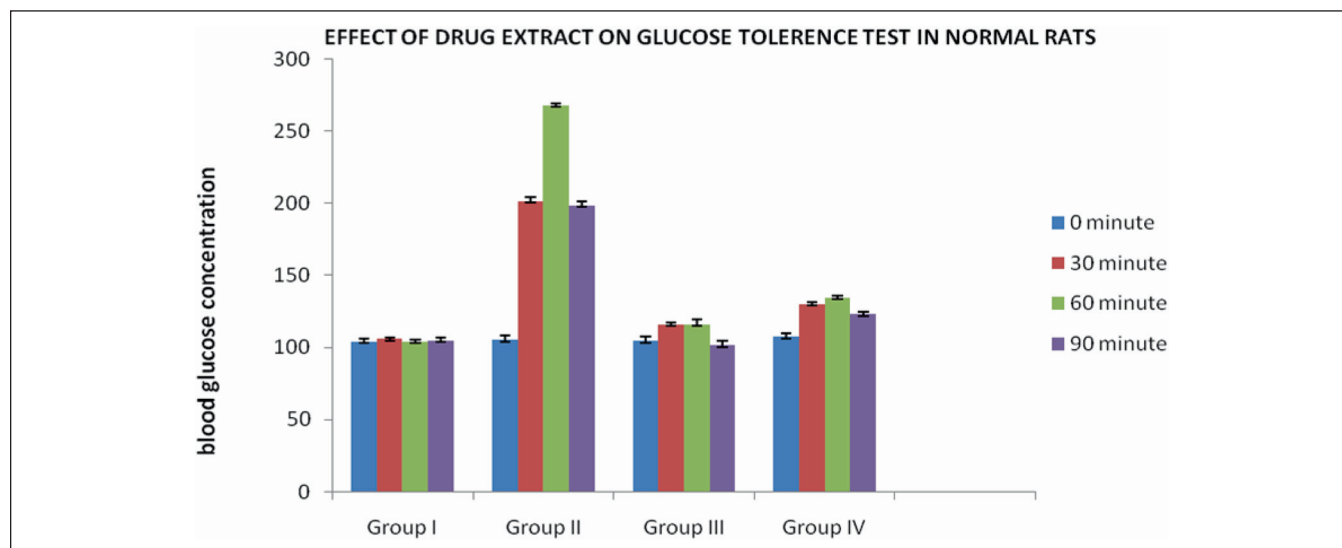


Figure 1: Effect of drug extract on glucose tolerance test in normal rats

The animals for Alloxan-induced diabetic study were randomly assigned into four groups (1-4) of six rats ($n = 6$) each as follows, namely

Group 1 - Received only normal saline orally

Group 2 - Diseased animals who received only alloxan monohydrate (120 mg/kg i. p.) Diseased control

Group 3 - Diseased animals who first received alloxan monohydrate (120 mg/kg body weight) once and then metformin (standard drug) 100 mg/kg body weight for 21 days.

Group 4 - Diseased animals treated methanolic extract of *Feronia elephantum* Corr. 500 mg/kg body weight for 21 days.^[17]

Determination of blood glucose levels

Blood samples were collected by cutting the tail-tip of the rats, for blood glucose determination at intervals of 1st, 7th, 14th and 21st day. Determination of the blood glucose level was done by the glucose-oxidase principle using the ONE TOUCH Basic (Lifescan, Milpitas, CA) instrument and results were reported as mg/dl.

Statistical analysis

Blood glucose levels were expressed in mg/dl as mean \pm SEM (standard error of mean). The data were statistically analyzed using one way ANOVA by Dunnett test. The comparison was made reference group and test group versus disease control group. The values of $P < 0.05$ and $P < 0.01$ were considered as significant.

RESULTS AND DISCUSSION

Phytochemical analysis

Freshly prepared extract were subjected to preliminary phytochemical screening test for various constituents. This revealed the presence of tannins, coumarins, flavonoids and essential oils (terpenoids) mainly. Further thin layer chromatography studies confirmed the presence of these phytoconstituents in the extract.

Pharmacological screening

Determination of acute toxicity and LD_{50} values

After using various doses level in various groups the toxicological data and LD_{50} values was determined for extract.

Table 2: Effect of extract on blood glucose level in alloxan Induced diabetic rats

Group	Dose	Blood glucose level (mg/dl) (mean \pm SEM)			
		1 st day	7 th day	14 th day	21 st day
Group I (normal control)	-----	97.62 \pm 2.72	98.560 \pm 1.77	91.920 \pm 02.18	87.340 \pm 02.18
Group II (disease control)	120 mg/kg	194 \pm 11.09	210.1 \pm 7.48	235.2 \pm 4.52	240.3 \pm 9.82
Group III (standard)	100 mg/kg	167.54 \pm 3.67*	165.85 \pm 2.54*	132.20 \pm 2.47*	115.25 \pm 2.23*
Group IV (Test)	500 mg/kg	180.30 \pm 5.2*	175.70 \pm 1.7*	145.65 \pm 5.76*	130.9 \pm 6.70*

Group I - normal control; Group II - Diseased control Group III - Diseased animals treated with standard drug Metformin; Group IV - Diseased animals treated methanol extract of *Feronia elephatum* Corr. 500 mg/kg

Values are expressed in Mean \pm SEM ($n = 6$), $P^* < 0.01$ when compared to Group II

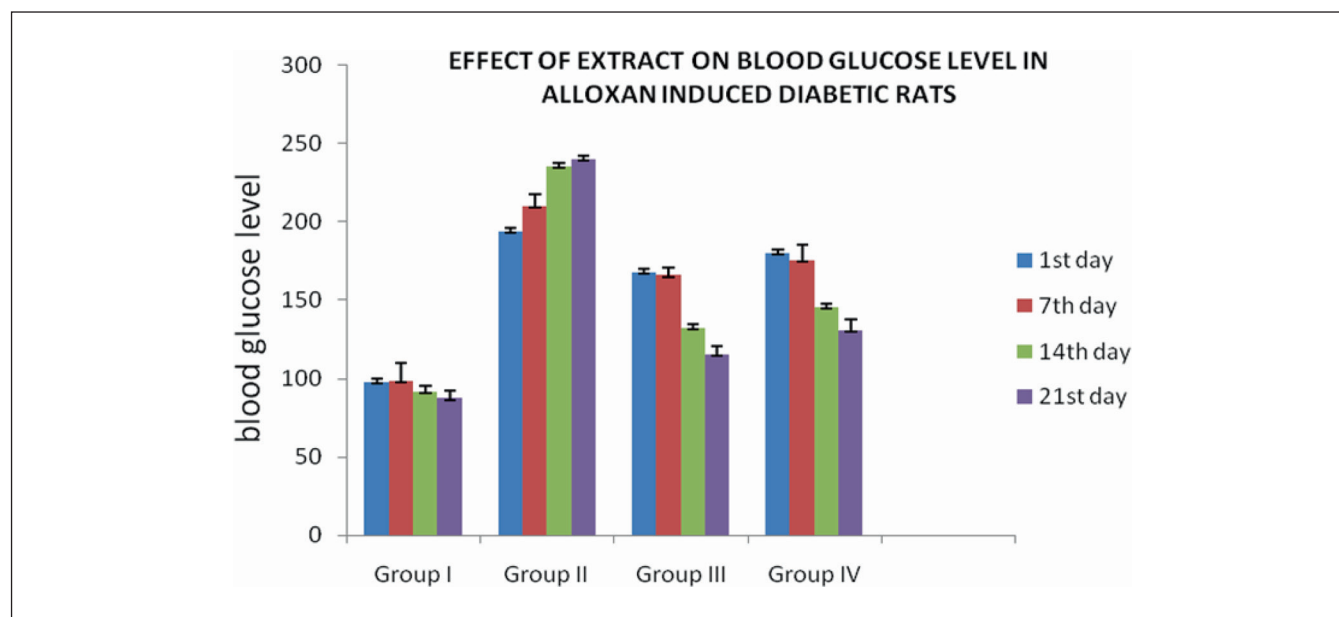


Figure 2: Effect of extract on blood glucose level in alloxan induced diabetic rats

No mortality was seen upto doses as high as 5 g/kg by staircase method. So a dose well below the possibly toxic (approximately 1/10th) of 500 mg/kg was taken.

DISCUSSION AND CONCLUSION

This study firstly evaluated the hypoglycemic effect of *Feronia elephantum* Corr. in glucose induced hyperglycemia and alloxan induced diabetic rats. It was found that pretreatment of *Feronia elephantum* methanolic extract in normal rats at a dose level of 500 mg/kg body weight caused a partial prevention of hyperglycemia induced by glucose (1.75 g/kg body weight). Methanol extract significantly reduced the blood glucose level after 90 minute of administration when compared to control group. In alloxan induced diabetes rat *Feronia elephantum* methanolic extract showed a significant decrease in blood glucose level when treated for 21 days at a dose level of 500 mg/kg body weight.

It was also observed that *Feronia elephantum* Corr. fruit pulp methanolic extract when administered to alloxan induced

diabetic rats, the weight loss was reversed and the animal returned to near normal when compared to disease control group. The ability of the methanolic extract of fruit pulp to protect body weight loss seems to be due to its ability to reduce hyperglycemia.

The possible mechanism by which fruits bring about a decrease in blood sugar level may be by potentiation of the insulin effect of plasma by increasing either the pancreatic secretion of insulin from β cells of the islets of Langerhans or its release from the bound form. A number of other plants have been reported to exert hypoglycemic activity through insulin release-stimulatory effect.

To our knowledge, this is the first study in which methanolic extract of fruits pulp of *Feronia elephantum* Corr. was proved *in vivo* a potent hypoglycemic/anti-hyperglycemic agent and this information can be useful for the management of Type-I as well as Type-II diabetes mellitus. On the basis of this study further research works are needed to understand the exact mechanism of action of hypoglycemia produced by drug and to isolate the moieties responsible for the activity.

Table 3: Body weight of the animal during the experiment in alloxan induced diabetic rats

Groups	Initial Body Weight	Final Body Weight
Group I (normal control)	162.47 \pm 3.98	179.67 \pm 3.42
Group II (disease control)	165.24 \pm 6.74	125.87 \pm 6.67
Group III (standard)	164.34 \pm 6.45	158.23 \pm 4.98
Group IV (Test)	170.54 \pm 7.60	142.21 \pm 3.77

Group I - normal control; Group II - Diseased control; Group III - Diseased animals treated with standard drug Metformin Group IV - Diseased animals treated methanol extract of *Feronia elephantum* Corr. 500 mg/kg
Values are expressed in Mean \pm SEM (n = 6),

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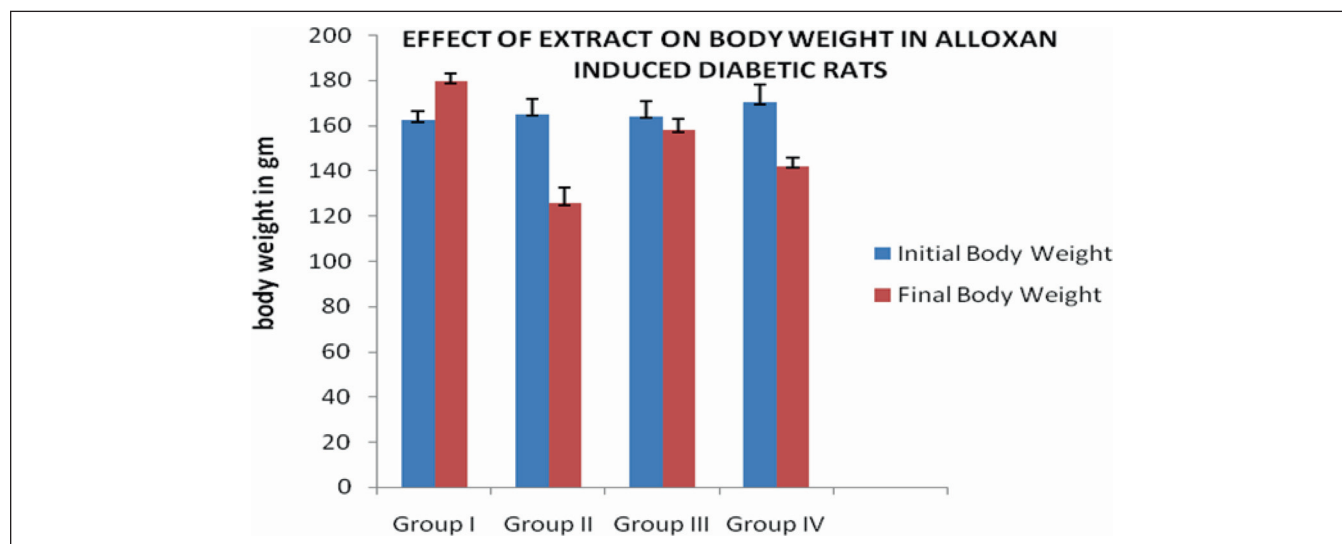


Figure 3: Effect of extract on body weight in alloxan induced diabetic rats

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