

**HYPOGLYCEMIC ACTIVITY OF AQUEOUS LEAF EXTRACT OF *FERONIA ELEPHANTUM* IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS**

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**Summary**

The hypoglycemic activity of the aqueous leaf extract of *Feronia elephantum* (Family: *Rutaceae*) was evaluated. The extract was administered orally 500 mg/kg to normal and streptozotocin-induced (60 mg/kg body weight, i.v) diabetic rats. The hypoglycemic effect of the extract was statistically significant with an oral dose of 1000 mg/kg and was comparable to that of the effect produced by standard antidiabetic agent, glibenclamide 0.9 mg/kg. In the oral glucose tolerance test, the extract increased the glucose tolerance.

**Key words:** *Feronia elephantum*, Glucose tolerance test, Hypoglycemic activity, Streptozotocin

**Introduction**

Plants provide a major resource for a large number of traditional medicines that have been in existence for thousands of years in country like India. Ayurveda, one of the oldest medicinal systems in the world, provides leads for a vast number of therapeutically useful compounds. The combination of traditional and modern knowledge can produce better source of the active constituents for the treatment of diseases with fewer side effects. Therefore, it is desirable to tap all the natural resources including plants to develop safer, effective and yet cheaper remedies for the treatment of chronic disorders like diabetes mellitus. Diabetes mellitus being major public health problem in the world and its incidence is increasing in India marketing it “diabetes capital of the world”<sup>1</sup>. The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025<sup>2</sup>. The most disturbing trend is the shift in age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation’s health and economy<sup>1</sup>.

Following the WHO's recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus, investigations on hypoglycemic agents derived from medicinal plants have also gained momentum<sup>3</sup>. The phytochemicals, identified from traditional medicinal plants are presenting an exciting opportunity for the development of new types of therapeutics in combating diabetes and diabetes-related complications. In addition several plant part extracts, routinely consumed vegetables like *Brassica oleracea*<sup>4</sup> and fruit and seeds of *Semicarpus anacardium*<sup>5</sup> have been reported to possess hypoglycemic activity. Plants like *Ichnocarpus frutescens*<sup>6</sup> and *Salvadora oleoides*<sup>7</sup> used by traditional healers are found to be useful to that diabetes. *Feronia elephantum* is another plant, widely used to treat not only diabetes but several other clinical conditions by traditional healers in rural areas.

*Feronia elephantum* Corr. syn. *Limonia acidissima* L. (Family: Rutaceae) is distributed throughout India at an elevation of 450 m. In traditional medicine, bark, leaves, fruits, and gum of this plant are used<sup>8</sup>. Alkaloid, coumarins, flavanones, lignin, (-)-(2*S*)-5,3'-dihydroxy-4'-methoxy-6",6"-dimethylchromeno(7,8,2",3")-flavanone from the bark<sup>9</sup>, fernolin, aurapten, marmesin, bergapten and xanthotoxin from root<sup>10</sup> and methyl chavicol as a major compound has been reported from the essential oil<sup>11</sup> of *Feronia elephantum*. The oil extracted from the seeds has been reported to be useful in itching, skin diseases and leprosy<sup>12</sup>. Chemical contents of the seeds of *Feronia elephantum* have been reported to be palmitic, oleic, linoleic, linolenic, palmitoleic, steric acids,  $\beta$ -sitosterol and  $\beta$ -amysin<sup>13</sup>. Ethanolic extract of the fruits of *Feronia elephantum* has been reported to significantly lower the blood glucose level in experimental rats<sup>14</sup>. There is no scientific data available for hypoglycemic activity of the leaves of *Feronia elephantum*. Traditional healers widely use leaf decoction of *Feronia elephantum* to treat diabetes and therefore the present study was planned to evaluate the hypoglycemic activity of water extract of *Feronia elephantum* both in euglycemic and streptozotocin-induced diabetic rats.

## Materials and methods

### Collection of plant material

The leaves of *Feronia elephantum* were collected in the month of March 2009 from Ramadurga Taluka of Belgaum district. The plant materials were identified and confirmed by Dr. Harsha Hegde, Taxonomist, Regional Medical Research Centre (ICMR), Belgaum, India. A voucher specimen (No. RMRC-412) was deposited in the herbarium of Regional Medical Research Centre, Belgaum for future reference.

### Preparation of extract

The leaves of *Feronia elephantum* (FE) were shade dried and reduced to coarse powder by mechanical grinding. The powdered material was macerated in distilled water for seven days with 2 ml of chloroform to avoid any fungal or bacterial contamination. The mixture was sonicated in cold water for 30 minutes before filtration. The sonicated mixture was filtered using muslin cloth and the filtrate so obtained was lyophilized to get in powder form. The powdered FE extract was stored in deep freezer at -20°C for the experimental use.

### **Animals and housing condition**

The healthy male Wistar rats (120-150 g body weight) used in the present study, were fed on commercial laboratory animal feed (Amrut brand, Sangli) and water *ad libitum*. The rats were housed in the laboratory for a week for acclimatization under 12:12 hours natural light–dark cycle. The animals were starved overnight with water *ad libitum* prior to the day of experimentation. Ethical clearance was obtained from Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

### **Dose determination**

Based on the preliminary studies carried out on euglycemic animals, in the present study the dose of 500 mg/kg and 1000 mg/kg of the extract were selected. Rat equivalent dose 0.9 mg/kg of glibenclamide, a standard hypoglycemic used in the present study was calculated using conversion table devised by Paget and Barnes<sup>15</sup>.

### **Studies in euglycemic rats**

The animals were fasted for 18 hours prior to the experiment, and allowed to free access of water. Fasted rats were divided into three groups of six animals in each group. Group I served as vehicle control which received saline p.o. The group II received aqueous leaf extract orally 500 mg/kg and group III received glibenclamide 0.9 mg/kg. The glucose in the dose of 2g/kg was administered orally after 30 minutes of various treatments. Blood samples were collected from tail vein at 0, 30, 60, 90 and 120 minutes post dose to estimate blood glucose using glucometer<sup>16</sup> (One touch, sure step, Life Scan, Inc, Milpitas, CA 95035 USA).

### **Studies in streptozotocin induced hyperglycemic rats**

The animals were fasted overnight and 60 mg/kg streptozotocin (STZ) dissolved in freshly prepared citrate buffer (0.1 M, pH 4.5) was given intravenously in a volume of 1 ml/kg. After 6 hrs 200 mg of glucose was given orally to protect from hypoglycemia. After a gap of one day the fasting blood glucose was estimated with the help of standard glucometer and those having blood glucose more than 300 mg/dl were included in the study. Selected animals were divided in three groups (n=6, in each) to receive different treatments orally. Group I served as vehicle control which received saline p.o. The group II received aqueous leaf extract orally 500 mg/kg and group III received glibenclamide 0.9 mg/kg. The glucose in the dose of 2g/kg and blood samples were collected from tail vein at 0, 30, 60, 90 and 120 minutes after glucose challenge to estimate blood glucose<sup>16</sup>.

### **Statistical Analysis**

The data were expressed as Mean  $\pm$  SEM and analyzed by ANOVA followed by Dunnet's post hoc test and  $P \leq 0.05$  was considered significant.

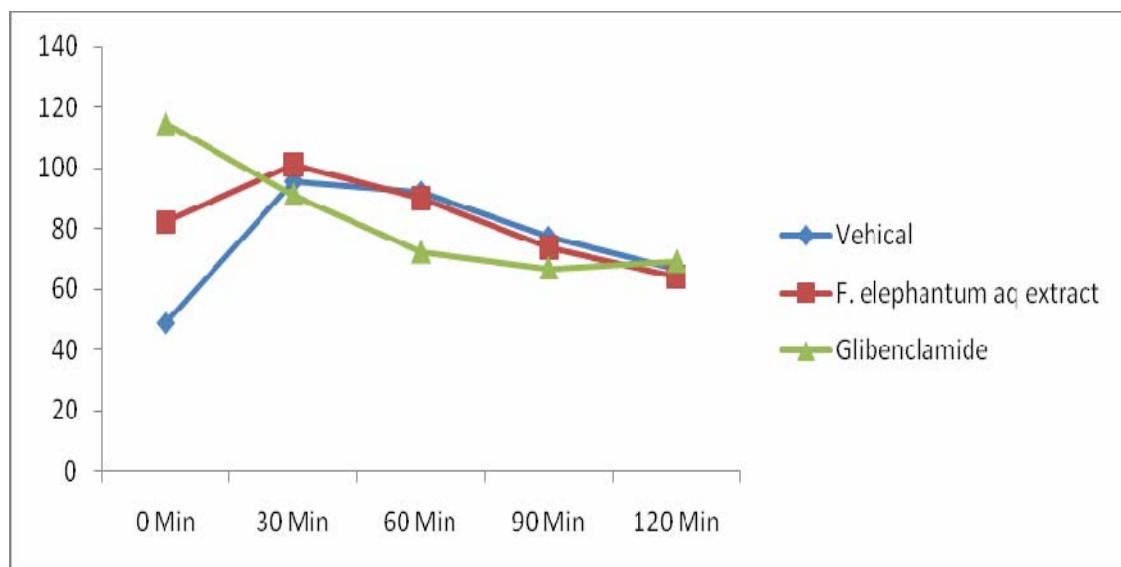
### **Results**

In OGTT, there was no significant change blood glucose levels of animals treated with FE extract as well as with glibenclamide as compared to those of vehicle treated controls (Fig. 1A and B). In STZ induced hyperglycemic rats, both the treatments lowered blood glucose as compared to that of control group, the reduction was not significant (Fig. 2 A and B).

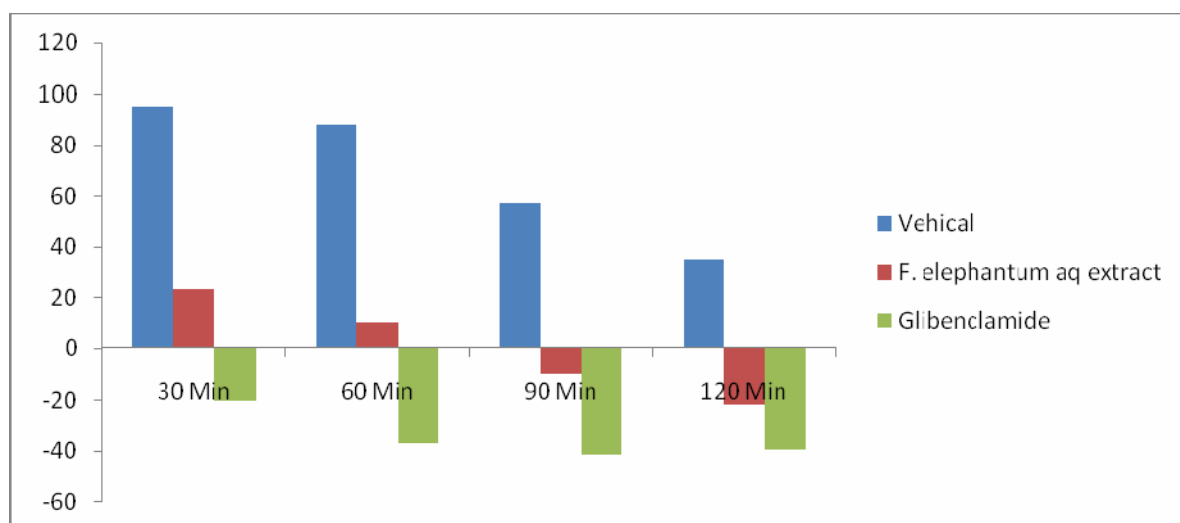
**Hyperglycemic rats:** As expected saline treatment did not alter the glucose level significantly, while glibenclamide in therapeutic equivalent dose decreased the blood glucose significantly at 3<sup>rd</sup> and 5<sup>th</sup> hr. FE extract treatment with the higher (1000 mg/kg) dose significantly lowered blood glucose at 3<sup>rd</sup> hr as compared to the 0 hr reading, indicating its hypoglycemic activity begins within 3<sup>rd</sup> hr after administration and disappears by 5<sup>th</sup> hr. (Table 1).

**Fig 1: OGTT in euglycemic rats treated with *Feronia elephantum* aqueous extract.**

**A: Mean change in blood glucose (mg/dl)**

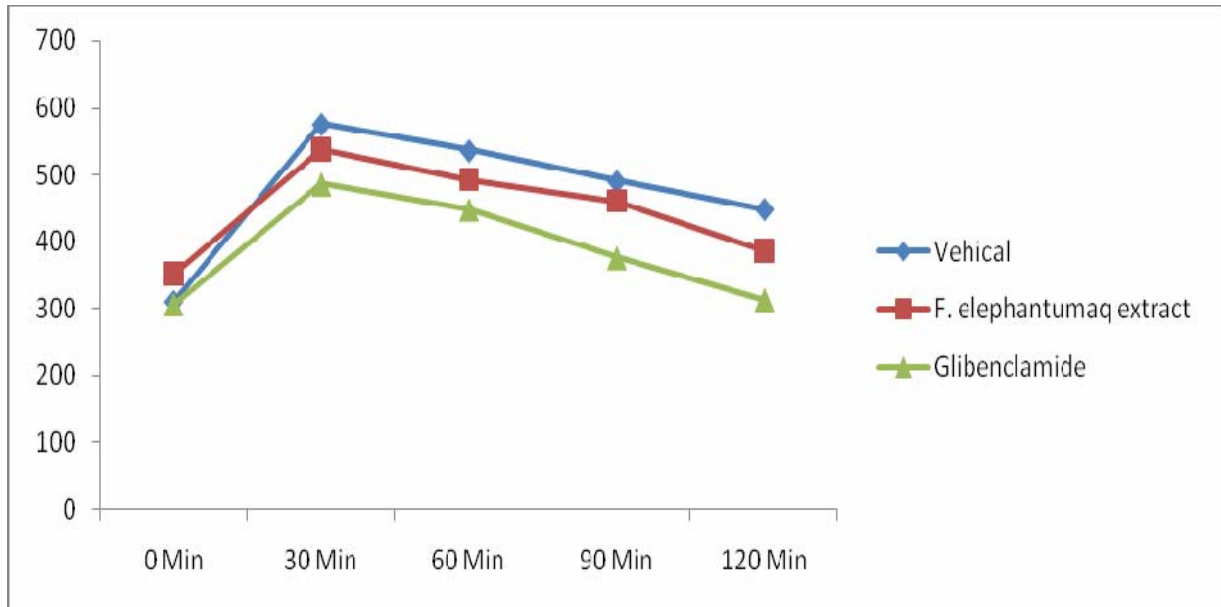


**B: Percent change in blood glucose**

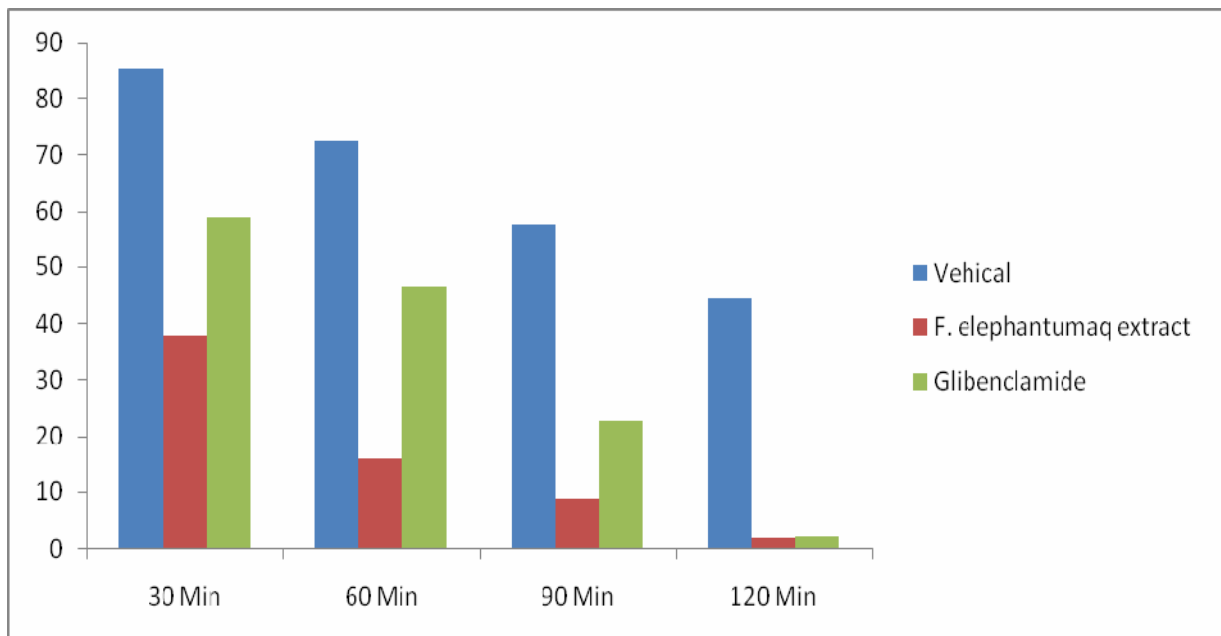


**Fig 2: OGTT in hyperglycemic rats treated with *Feronia elephantum* aqueous extract.**

**A: Mean change in blood glucose (mg/dl)**



**B: Percent change in blood glucose**



**Table 1. Effect Of *Feronia elephantum* aqueous extract on blood glucose of stz induced diabetic rats.**

Treatment groups ↓	Blood glucose mg/dl (Mean ± SEM)			
	0 hr	1hr	3hr	5hr
Vehicle	310.83 ± 23.04	317.61 ± 4.87	318.16 ± 3.34	320.83 ± 4.35
<i>F. elephantum</i> (1000mg/kg)	448.80 ± 25.81	404.60 ± 39.64	316.00 ± 29.50*	344.25 ± 35.66
Glibenclamide (0.9mg/kg)	322.8 ± 3.26	310.60 ± 4.2	303.00 ± 3.74*	261.40 ± 7.71**

\*\* = P<0.01, \* = P<0.05

### Discussion

Finding of the present preliminary investigation of the aqueous extract of *Feronia elephantum* in streptozotocin induced hyperglycemic rats showed a clear hypoglycemic activity, which is in accordance with traditional use of the plants leaves to reduced the blood glucose level though it was of short duration as compared to that of glibenclamide. This finding is in accordance with traditional use of the plant leaves in the form of decoction for diabetes. Probably repeated administration in higher dose of FE extract for a prolonged period may produce sustained hypoglycemic effect. To identify the main hypoglycemic constituents in the extract, to elicit its possible mechanism of hypoglycemic activity and to evaluate its efficacy on chronic administration, further studies are desirable.

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