Effect of ethanolic extract of *Feronia elephantum* Correa fruits on blood glucose levels in normal and streptozotocin-induced diabetic rats

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Abstract

_Feronia elephantum* Correa (Family — Rutaceae) commonly known as Wood apple has been widely used in Indian folk medicine for treatment of blood impurities, leucorrhoea, and as diuretic and liver tonic. It has not been studied experimentally for its blood glucose lowering potential earlier hence present study was conducted. Oral administration of 250mg/kg body weight of 95% ethanolic extract of unripe fruits significantly lowered the blood glucose levels of fasted, fed and streptozotocin-induced diabetic male albino rats. It also depressed the peak value in glucose loaded model. Further, study on histology of pancreatic β-cells granularity of normal rats was also done. Marked degranulation in β-cells of extract treated rats, associated with the blood glucose lowering was observed. Extract probably lowered the blood glucose concentrations by stimulating insulin secretagogue activity.

**Keywords:** Blood glucose lowering, β-cells, Ethanolic extract, _Feronia elephantum_, Streptozotocin-diabetes, Wood apple.

**IPC code, Int. cl.** — A61K 36/75, A61P 3/10

Introduction

In India, plant and herbs have been widely used by Ayurveda for the treatment of various ailments since vedic glory. Indian traditional medicine is indeed rich in a vast variety of flora, having ample scope in search of effective herbal remedy for diabetes¹. _Feronia elephantum* Correa [syn. _F. limonia_ (Linn.) Swingle]²-⁵ is a moderate size slow growing tree, with strait sharp strong spines. It is commonly known as Wood apple or _Kath-Bel_ and belongs to family—Rutaceae. Wood apple is indigenous in South India, Ceylon and Java and cultivated in many parts of India. The tree grows up to an elevation of 450 m in the Western Himalayas. Traditionally, it is consumed as fruit and vegetable in Sri Lanka⁶.

The fruits are sour and sweet, and used in India as liver and cardiac tonic. It is effective in treatment of diarrhoea, dysentery, hiccough, sore throat and gum diseases. In Ayurveda, it has been used for leucorrhoea and removing blood impurities and in Yunani medicine as diuretic³. The unripe fruits contain 0.015% stigmasterol and seeds contain oil high in saturated fatty acids⁷. An acidic heteropolysaccharide isolated from the _F. elephantum_ was reported to inhibit carcinoma cell growth⁸. Active compounds from stem bark were found to possess antimicrobial activity⁹. Oral administration of aqueous extract of leaves produced hepatoprotective activity at 400mg/kg and 800mg/kg in albino rats⁹. It has not been studied experimentally for its blood glucose lowering potential, thus in the present study we investigated the blood glucose lowering activity of 95% ethanolic extract of unripe fruits of _F. elephantum_ in normal and streptozotocin-induced diabetic rats, along with its effect on histology of insulin producing pancreatic β-cells granularity.

Materials and Methods

Unripe fruits of _F. elephantum_ (Fig. 1) were collected fresh in month of November from a village Bakshi Ka Taalab (latitude 26°59’ 46” N and longitude 80°54’ 9” E) of Lucknow following standard guideline given by Harischleger⁶. The plant was botanically identified and authenticated by Dr. Nirmala Upadhyay and a voucher specimen (LU 2/23560/06) was deposited in the Botany Department of Lucknow University. Unripe fruits were sliced, air dried in shade and powdered mechanically. Coarse powder of fruits was extracted by using 95% ethanol in Soxhlet extractor¹⁰. The extracted material was concentrated and an alcohol free semi-solid crude extract was obtained (wet fruits collected-5kg; yield of dry
weight/kg wet fruits-274.0g; crude extract yield/100g dried fruits weight-23.20g). Extract was orally fed to experimental group by metal canula at a single dose of 250mg/kg body weight as prepared in 2% gum acacia suspension and control was fed only 2% gum acacia11, 12. All the chemicals used were of analytical grades from E. Merk Ltd., West Germany and Sigma-Aldrich Chemicals, USA.

Male albino rats (120-140g) of Charles Foster strain were used and maintained on Hind Lever pellets diet (Mumbai, India), housed in polypropylene cages at a temperature of 23 ± 2°C and relative humidity 60 ± 5 with 12 h each of dark and light cycle. Water was allowed ad libitum. The rats were divided in to experimental and control groups of six rats each. Acute toxicity study13 of extract on behavioral aspect was carried out up to dose of 2500mg/kg body weight and found to be safe. The blood glucose lowering effect of the extract was examined in following four different experimental models14:

(a) **Fasted:** Blood was collected (at 0h) from the tail vein of the overnight (18h) fasted rats and extract was fed. Again blood samples were collected at 1, 3 and 4 hour interval after feeding extract. Blood glucose concentrations were estimated by Nelson’s Somogyi method15.

(b) **Fed:** Excess amount of pellets were put in the cages on previous evening, so that some pellets were found left over in the next morning. Blood was collected before (at 0h) and after administration of the extract at 1, 3 and 4 hour interval for glucose estimation.

c) **Glucose loaded:** Animals were fasted for 18 h and blood was collected (at 0h) for glucose estimation. Now extract was fed and after half an hour glucose (1.5g/kg b.w. oral) solution was administered and blood samples were collected for glucose estimation at 1/2, 1 and 3 hour interval.

d) **Diabetic:** Diabetes was induced in rats by injecting streptozotocin (35mg/kg b.w.) dissolved in the chilled citrate-phosphate buffer (pH 4.5) through tail by the method of Theodorou et al16. At the moment of injection the rats had fasted for 18 hours. After the streptozotocin injection, all rats were returned to their cages and given free access to food and water. Two to three days after injection, diabetes was confirmed by checking urine glucose by Diastex17. Then the experiments were performed using identical procedure as in the fasted model (a).

**Statistical analysis of data**

Mean and standard error were determined and students’ t’ test was performed between experimental and control group. The results were considered statistically significant, if the p values were 0.05 or less.

**Histology of Pancreas**

Pancreas of anesthetized animals of extract treated and control group were taken out at the most effective hours associated with blood glucose lowering and fixed in alcoholic Bouin’s fluid. Paraffin sections of pancreas were double stained with haematoxylin and eosine, and granularity of insulin producing β-cells was compared under microscope18, 19.

**Results**

In fasted group of rats ethanolic extract of unripe fruits produced significant (P<0.05) blood glucose lowering at 3h as compared to control (Table 1). In extract treated group, maximum percentage of blood glucose lowering (16.29% at 3h) from the initial value (0h) was higher as compared to control (5.42% at 4h). Ethanolic extract produced significant blood glucose lowering in fed rats at 3h (P<0.01) as compared to corresponding control. Maximum percentage lowering of blood glucose from the initial value (0h) was higher in extract treated group (13.98% at 3h) than control (7.08% at 4h).

In diabetic group of albino rats extract of unripe fruits produced significant (P<0.05) lowering of blood glucose at 3h and 4h after the extract administration. Maximum percentage of blood glucose lowering in extract treated group from the initial value (0h) was 11.26% at 3h, but no lowering recorded in control.

Ethanolic extract of unripe fruits produced significant (P<0.05) suppression of blood glucose level at 1 and 3h in glucose loaded model as compared to control (Table 2). In terms of maximum percentage of blood glucose rise from the initial value (0h), the extract was exhibited more blood glucose lowering effect (18.05% rise at 1h) than 2% gum acacia in control group (33.98% rise at 1h).

During the histological examination of pancreas of extract treated fasted (Fig. 2B) and fed (Fig. 2D) rats,
degranulated β-cells were observed at 3 h post-treatment as compared to their respective controls (Figs 2A and 2C), associated with the blood glucose lowering. In glucose loaded rats also, the marked degranulation in β-cells was observed at 3 h post-treatment (Fig. 2F) as compared to corresponding control (Fig. 2E).

**Discussion**

In the present study, four test models having different background of blood glucose levels, that resemble some patho-physiological condition of the body have been used to increase the sensitivity of experimental procedure and to spot blood glucose lowering activity of *F. elephantum* with certainty\(^{14}\). Male albino rats were chosen as experimental animal because, their blood glucose levels are fairly stable and have been most widely used in such studies, leading to generation of adequate baseline data\(^{11}\). The ethanolic extract was orally administered as this route was thought to be most preferable and in tune with the route of administration of 250mg/kg b.w. dose as reported earlier by various workers\(^{11,12}\). In the present study, 95% ethanolic extract of unripe fruits was found to produce significant lowering of blood glucose levels in fasted, fed and streptozotocin-induced diabetic male albino rats. Extract was also improved the oral glucose tolerance. In the present experiments, blood glucose lowering effect induced by fruits extract, persisted for 3 to 4 h after feeding, hence *F. elephantum* definitely lowers the blood glucose in rats. In extract treated normal rats, reduction in blood glucose levels is associated with the degranulation in pancreatic β-cells. It suggests that the rate of extract induced insulin release from β-cells is probably greater than the rate at which β-cells replenish their insulin stores.

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Fig. 1: *Feronia elephantum* fruits

Fig. 2 (A-F): Showing, rich granulation in β-cells of islets of Langerhans of control group (A) Fasted, (C) Fed, (E) Glucose loaded, and marked degranulation in β-cells of the extract treated group (B) Fasted, (D) Fed, (F) Glucose loaded at 3 h post-treatment (Magnification-63X)
Table 1: Effect of 95% ethanolic extract of *Feronia elephantum* fruits at a single oral dose of 250mg/kg body weight, on blood glucose levels of fasted, fed and streptozotocin-induced diabetic male albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose level mg/100ml (Mean ±S.E.) at time (hours)</th>
<th>Maximum % of blood glucose lowering from initial value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1h</td>
<td>3h</td>
</tr>
<tr>
<td>Fasted</td>
<td>Control</td>
<td>83.38±4.78 (6)</td>
<td>82.80±2.94 (6)</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>85.08±3.18 (6)</td>
<td>84.27±3.19 (6)</td>
</tr>
<tr>
<td>Fed</td>
<td>Control</td>
<td>103.65±4.23 (6)</td>
<td>100.14±2.10 (6)</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>103.36±2.16 (6)</td>
<td>94.94±3.08 (6)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>Control</td>
<td>189.55±6.91 (6)</td>
<td>198.64±7.24 (6)</td>
</tr>
<tr>
<td></td>
<td>EG</td>
<td>195.34±5.14 (6)</td>
<td>181.52±8.93 (6)</td>
</tr>
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</table>

Significance between the control and EG (experimental group): *P*<0.05, *P*<0.01; In parenthesis the number of rats used is given.

Table 2: Effect of 95% ethanolic extract of *Feronia elephantum* fruits at a single oral dose of 250mg/kg body weight, on glucose tolerance (1.5g/kg b. w. orally) of male albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose level mg/100ml (Mean ±S.E.) at time (hours)</th>
<th>Maximum % of blood glucose rise from initial value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>1/2h</td>
<td>1h</td>
</tr>
<tr>
<td>Glucose</td>
<td>Control</td>
<td>74.71±2.07 (6)</td>
<td>94.55±4.63 (6)</td>
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<tr>
<td></td>
<td>EG</td>
<td>73.65±2.27 (6)</td>
<td>84.74±3.07 (6)</td>
</tr>
</tbody>
</table>

Significance between the control and EG (experimental group): *P*<0.05, *P*<0.01; In parenthesis the number of rats used is given.

**Conclusion**

Thus, we can conclude that ethanolic extract of *F. elephantum* unripe fruits probably have insulin secretagogue activity that lowered the blood glucose levels in male albino rats. Further, investigations on *F. elephantum* are needed to isolate the active principle(s) responsible for such activity and find out their effect in long term studies.

**Acknowledgement**

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**References**


