Research Article

Evaluation on antidiabetic activity of hydroalcoholic extract of bark of *feronia limonia*

Nandini Hingwasia[1], Salaj Khare[1*], B K Dubey[2], Amit Joshi[1], Suresh Dhakad[1], Amit Jain[1]

[1]*TIT- Pharmacy Education and Research, Anand Nagar, Bhopal, M.P. India*

[2]*Technocrats Institute of Technology- Pharmacy, Anand Nagar, Bhopal, M.P. India*

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Abstract

Objective: The aim of the present study is to evaluate the anti-diabetic activity of hydroalcoholic extract of bark of *Feronia limonia* on alloxan induced rats. The plant belongs to family Rutaceae , is an angiosperm with various medicinal properties including anti-diabetic, anti-bacterial, anti-inflammatory, hepatoprotective, anti-oxidant, muscle relaxant, larvicidal and anti-tumour. Bark extract principally showed the presence of flavonoids and phenols. **Materials and methods:** Alloxan (120 mg/kg) was dissolved in saline and administered intravenously into fasted rats. The animals were divided into 4 groups randomly consisting of 6 rats in each group as follows: group 1 (normal control) includes non- alloxanized rats (0.5% normal saline, 5 ml/kg body weight orally for 21 days), group 2(positive control) untreated diabetic control (0.5 ml/100 gm normal saline), group 3 (standard) diabetic rats treated with reference drug glibenclamide 5 mg/kg p.o. for 21 days and group 4 (test) diabetic rats treated with test drug 300 mg/kg for 21 days. **Results:** At the end of 21st day, blood glucose level and lipid profile was found to be effectively reduced as compared to glibenclamide. Histopathological study was carried out which reveals disturbances in pancreatic cells i.e. positive control group was found to have pronounced edema whereas normal group resembles test in having minimum edema. **Conclusion:** Thus, it can be concluded from above that *Feronia limonia* bark extract is found to be efficacious in reducing BGL and lipid levels.

Keywords: *Feronia limonia,* Rutaceae, anti-diabetic, flavonoids, glibenclamide

Introduction

Diabetes Mellitus is a syndrome with disordered metabolism and inappropriate hyper glycemia due to either a deficiency of insulin secretion or to a combination of insulin resistance and inadequate insulin secretion to compensate for the resistance. Its complications include heart attack, stroke, cataracts, glaucoma, diabetic foot, peripheral artery disease, peripheral neuropathy, diabetic nephropathy and retinopathy (Papadakis et. al., 2015; Waugh and Grant, 2010).

Wood Apple botanically known as *Feronia limonia* is the only species in its genus belonging to family Rutaceae, found in the tropical and sub-tropical regions of the world. The plant is an angiosperm with multi-faceted medicinal properties almost every part of it is used traditionally. The various medicinal properties include anti-diabetic, anti- bacterial, anti-inflammatory, hepatoprotective, anti-oxidant, muscle relaxant, anti-histaminic, wound healing, larvicidal and anti-tumour. Being a fruit of tropical regions, it requires dry arid conditions for growth with certain amount of monsoon. In India, it is cultivated in the states such as Maharashtra, Tamil Nadu, Madhya Pradesh, Karnataka, Andhra Pradesh, Kerala and certain regions of western Himalayas. Maharashtra, being the largest producer. It is also cultivated in Bangladesh, Pakistan and Sri Lanka (Amin H et. al., 2017).

The literature survey revealed that the bark extract of *Feronia limonia* was not earlier studied experimentally for its blood glucose lowering potential thus the present study was carried out to investigate the blood glucose lowering activity of hydroalcoholic bark extract of *Feronia limonia* in normal and alloxan induced diabetic rats and the antidiabetic activity is compared with the standard drug Glibenclamide. The determination of its lipid lowering effect and histopathology study of pancreas was also performed.

Materials and methods

Plant material and extraction

The selected plant was collected in sufficient quantity from the garden premises of TIT College in Bhopal (MP) in the month of June. The plant material was authenticated by Dr.

**Address for Corresponding Author:*
Salaj Khare
TIT- Pharmacy Education & Research, Anand Nagar, Bhopal-462021, (M.P.) India.
Email: salajkhare@rediffmail.com; Phone: 9617774991

Zia ul Hasan (HOD-Dept. of Botany) from Saifia Science College, Bhopal and the Boucher no. for the specimen is 451/Bot/Saifia/17.

The barks of *Feronia limonia* were dried under shade in laboratory. They were pulverized to make coarse powder. The coarse powder of seeds was passed through sieve No.18 to maintain its uniformity and stored in cool and dry place for further study.

Extraction was done using Soxhlet Apparatus. This is a continuous process of extraction with a hot solvent. The hydroalcoholic solvent system is used in the ratio 1:1 and the temperature was set at 60-70°C. After 72 hours, extract was dried at room temperature. Crude dried extract was then preserved in air tight containers such as a dessicator or in a freezer.

Experimental animals

Male and female Wistar albino rats (150-250g) were provided by Sapience Bioanalytical Research Lab., Bhopal, and Madhya Pradesh, India. The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 44 –56% and light and dark cycles of 12:12 hours, fed with standard pellet diet and water).

Chemicals

Alloxan monohydrate, Glibenclamide (Std drug) and other reagents used in the experiment were obtained from laboratory and were of analytical grade.

Acute oral toxicity study

Acute oral toxicity study was evaluated as per OECD guidelines (423) on Wistar albino rats in the experiment. Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression, and mortality after dosing for 12 days consecutively. Administered dose was found tolerable (as no death found). Therefore, two dose levels 200 mg/kg& 300 mg/kg were selected for anti-diabetic activity (Roll et. al, 1986).

Induction of diabetes in rats

Alloxan monohydrate was dissolved in saline and administered intravenously into fasted rats at a dose of 120 mg/kg body wt (Rohilla A et. al., 2012). The rats were given 5% (w/v) glucose solution in feeding bottles for next 24 h in their cages to prevent hypoglycaemia after alloxan injection. After 72 h rats with BGL greater than 200 mg/dl and less than 400 mg/dl were selected and observed for consistent hyperglycaemia (fasting blood glucose level greater than 200 mg/dl and lesser then 400 mg/dl) upto 7 days.

The treatment was continued for the next 21 days and blood samples were collected on 0th, 7th, 14th and 21st days after 1 hr administration. Blood glucose level (BGL) was estimated with the help of U.V. by ERBA diagnostic Glucose kit.

Randomization, grouping and dosing of animals

The animals were divided into four groups randomly consisting of 6 rats in each group as follows:

GROUP 1: Normal Control-Non-alloxanized rats (0.5% normal saline, 5 ml/kg body weight orally for 21 days (p.o.))

GROUP 2: Positive Control - Untreated diabetic control (0.5 ml/100gm normal saline)

GROUP 3: Standard - Diabetic rats treated with reference drug glibenclamide 5 mg/kg p.o. for 21 days

GROUP 4: Test - Diabetic rats treated with test drug 300 mg/kg for 21 days

Triglyceride (TG) Determination

Pipette into 3 tubes working reagent, add distilled water to 1st, Standard to second and test sample to 3rd pipette simultaneously. Mix and incubate for 10 min at 37°C. Read the absorbance of standard and each test at 505 nm on biochromatic analyser against reagent blank.

$$\text{TG}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{Abs of test}}{\text{Abs of standard}} \times \text{concentration of standard mg/dl}$$

High density lipoprotein (HDL) Determination

a) Precipitation: It involves use of sample and HDL reagent in definite proportions. Mixed well, allow to stand for 10 min at room temp, mix again and centrifuge for 10 min at 4000 rpm. After centrifugation, separate the clear supernatant from the ppt within 1 hr and determine the HDL cholesterol concentration using cholesterol reagent.

b) HDL cholesterol determination: involves the use of cholesterol reagent, standard (HDL) and HDL supernatant. Mix and incubate for 5 min at 37°C. measure the absorbance of the standard and sample against the reagent blank.

$$\text{HDL Cholestrol}\left(\frac{\text{mg}}{\text{dl}}\right) = \frac{\text{Abs of sample}}{\text{Abs of standard}} \times \text{concentration of standard x2}$$

Where, 2 = dilution factor of the sample

Histopathological study

Pancreas of control, standard and extract treated animals were isolated for histopathological examination. Isolated pancreas after washing in PBS solution is stored in 10% Formalin. Paraffin sections of pancreas tissue were stained

in haematoxylin and eosin for evaluation of β cells of islets in light microscope (David S K et. al., 1991).

Statistical analysis

The results are expressed as mean ±SD. Statistical difference between normal and diabetic groups were determined using one-way analysis of variance (ANOVA) followed by Student's t- test. A difference in the mean p-value<0.01 was considered as significant.

Results

Phytochemical Analysis

The bark extract obtained from *Feronia limonia* was subjected to phytochemical screening which reveals the presence of pharmacological active compounds such as alkaloid, glycosides, saponnins, flavonoids, tannins and phenols (Ko et. al., 1998; Khandelwal, 2000; Kokate, 2010).

Blood glucose level

Treatment of the rats with alloxan resulted in approximately 2-3-fold increase in blood glucose concentration in comparison to normal control rats. The effect of dose of *Feronia limonia* bark extract in lowering blood glucose level were shown in table below where blood glucose levels was found to be decreasing simultaneously. Results are shown in table 1.

Lipid profile

The lipid level i.e. cholesterol, triglycerides, high density lipids and low density lipids of the animals in each group especially in test is found to be decreasing with the ongoing treatment process. Results are shown in table 2.

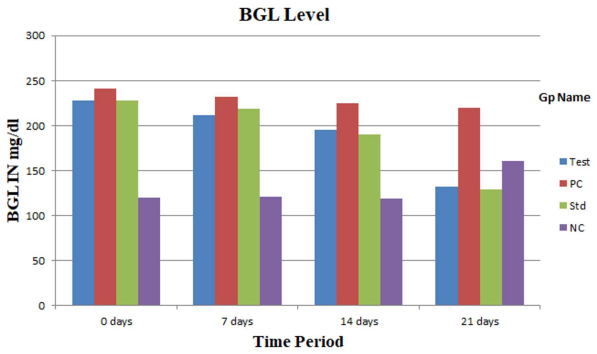

Figure 1. Effect of hydroalcoholic extract of bark of *feronia limonia* on blood glucose level

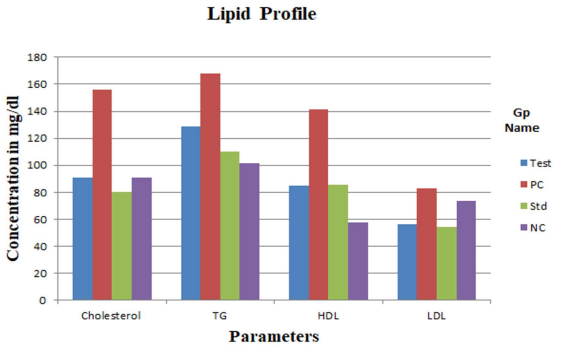

Figure 2. Effect of hydroalcoholic extract of bark of *feronia limonia* on lipid level i.e. cholesterol, triglyceride, high density and low density lipids

Histopathological study

Histopathological study was carried out which reveals disturbances in pancreatic cells i.e. positive control group was found to have pronounced edema whereas normal

Table 1. Effect of hydroalcoholic extract of bark of *feronia limonia* on blood glucose level

Group Name	0 days	7 days	14 days	21 days
Test	228.00±8.029	211.50±5.506	195.67±7.351	132.17±2.496
PC	241.00±7.151	232.17±5.173	224.50±5.835	220.16±5.40
Std	227.67±4.379	218.50±4.794	190.66±6.606	128.83±1.701
NC	119.67±3.127	120.50±3.528	119.00±3.587	160.84±4.438

Results are expressed as mean ± SEM (n=06), Dunnet's multiple comparisons test

Table 2. Effect of hydroalcoholic extract of bark of *feronia limonia* on lipid level i.e. cholesterol, triglyceride, high density and low density lipids

Group Name	Cholesterol	TG	HDL	LDL
Test	90.83±5.15	128.66±6.71	85.00±3.79	56.00±5.89
PC	156.00±8.91	168.14±10.99	141.66±2.12	82.66±2.81
Std	80.50±2.60	109.83±3.61	85.66±2.99	54.00±4.84
NC	91.00±4.48	101.83±5.21	57.83±3.88	73.53±7.92

Results are expressed as mean ± SEM (n=06), Dunnet's multiple comparisons test

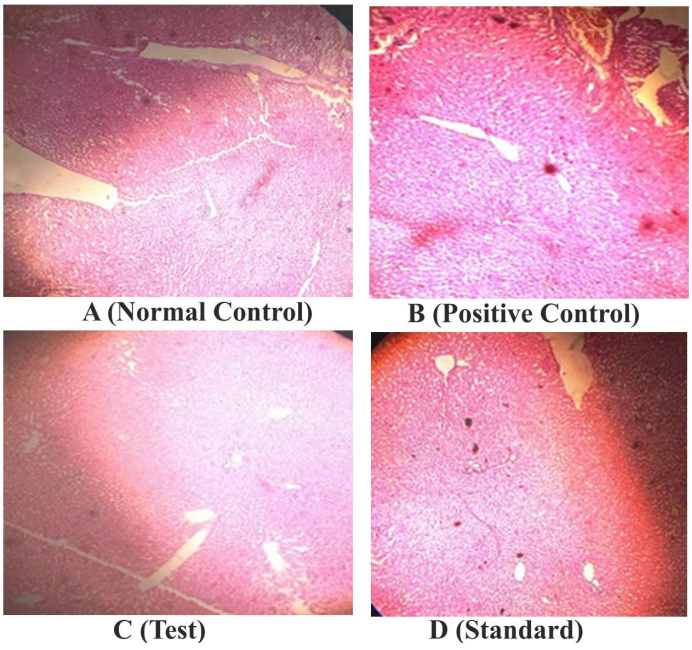

Figure 3. Histopathological observations of pancreas after treatment with hydroalcoholic extract of bark of *feronia limonia* **Normal control**: Pancreatic section showed normal pancreatic structure of cells (normal islets of langerhanns and normal acini tissues. They were found to have minimum edema.; **Positive Control**: Pancreatic cells showed disorganisation of cellular structure representing degenerative and necrotic changes. They were found to have pronounced edema.; **Standard**: Pancreatic cells showed marked improvement and gradual restoration.; **Test**: Pancreatic cells treated with bark extract showed healthy structure and gradual restoration of cells with minimum edema (similarity with normal control cells).

group resembles test in having minimum edema. Results are shown in figure 3.

Discussion

In the present study, extract of *Feronia limonia* bark was studied for anti-diabetic activity at the concentration (350mg/kg) in alloxan induced diabetic rats. Administration of alloxan (120mg/kg) caused rapid destruction of β cells which lead to reduction in insulin release and impaired glucose uptake by pancreatic cells and tissues. The results of the present study indicated significant glucose lowering effect of *Feronia limonia* bark extract in alloxan induced diabetic rats. Percentage reduction in blood glucose level of extract treated animal increases gradually from day 1 onwards. Maximum fall of 57% at the end of 21st day of treatment with *Feronia limonia* extract (350mg/kg) is observed. Glibenclamide being the standard drug for type 2 diabetes act by stimulating the β cells of pancreas to release insulin. Standard drug produced the maximum fall of 56% at the end of 21st day of treatment. Literature survey revealed flavonoids and phenols are effective anti-hyperglycaemic agents which can regenerate the β cells in alloxan induced diabetic rats. In the present study, preliminary phytochemical screening of extract showed the presence of flavonoids, phenols, alkaloids, saponnins and glycocides. Induction of diabetes by alloxan leads to slight loss of body weight due to increase muscle wasting and loss of tissue proteins. These diabetic rats regained its original body weight on treatment with extract for 21 days. The result from the present study also reported significant decrease in serum cholesterol levels in diabetic rats treated with bark extract. Hence, *Feronia limonia* extract proves its efficacy in reducing both glucose levels and lipid levels.

Conclusion

Findings of the present study, on the basis of exhaustive literature review and preliminary phytochemical screening clearly indicates that treatment with bark extract of *Feronia limonia* showed significant hypoglycaemic activity. As elucidated, the objective of the study was to investigate the hypoglycaemic activity of hydro-alcoholic extract of *Feronia limonia* and was not aimed to explain the mechanism of anti-diabetic activity of the extract. Literature survey revealed flavonoids and phenols are effective anti-hyperglycemic agents which can regenerate the damaged β cells in alloxan induced diabetic rats. The results from the present study also indicate significant decrease in serum cholesterol level of diabetic rats treated with extract compared to diabetic control. Presence of pharmacological active compound in the plant can be useful for further future research. However, the result suggest that bioactive constituents responsible for improving type 2 diabetes in rats needs to be isolated, characterized and explored clinically and experimentally.

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Conflicts of interest: The authors have no conflicts of interest.

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