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## **Antidiabetic Activity of *Feronia limonia* and *Artocarpus heterophyllus* in Streptozotocin Induced Diabetic Rats**

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### **ABSTRACT**

Antidiabetic activity of *Feronia limonia* Fruit and *Artocarpus heterophyllus* Bark extracts used as edible medicine by local tribal population of Vellore districts were analyzed in streptozotocin induced diabetic rats. Preliminary phytochemical screening revealed the presence of high contents of flavonoid in methanolic extract of *Feronia limonia* and ethyl acetate extract of *Artocarpus heterophyllus* compared to other extracts. Diabetes was induced by single intraperitoneal injection of streptozotocin (45 mg kg<sup>-1</sup>). Diabetic rats were treated with these extracts at a dose of 200 and 400 mg kg<sup>-1</sup> for 30 days. Hypoglycemic activities of extract treated diabetic rats were assessed by the percentage reduction in fasting blood glucose level. *Feronia limonia* extract showed significant (p<0.05) decrease in blood glucose level when compared to the *Artocarpus heterophyllus* extract. Treatment of these extracts also had significant decrease in serum cholesterol level and regain in body weight of diabetic rats. The results suggest that unique bioactive constituents responsible for improving type 2 diabetic rats are present and further has to be purified, isolated and characterized to contribute better therapy for NIDDM.

**Key words:** *Feronia limonia*, *Artocarpus heterophyllus*, antidiabetic activity, streptozotocin

### **INTRODUCTION**

Diabetes mellitus describes a metabolic disorder of multiple etiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). Diabetes is divided into two major categories: Type 1 diabetes (Insulin-dependent diabetes mellitus or IDDM) usually develops in childhood and adolescence and patients require lifelong insulin injections for survival. Type 2 diabetes (Non-insulin dependent diabetes mellitus or NIDDM) usually develops in adulthood and is related to obesity, lack of physical activity and unhealthy diets. In the year 2004, according to the World Health Organization reports, more than 150 million people throughout the world suffered from diabetes of which 90% is type 2. Despite the presence of known anti-diabetic medicines in the pharmaceutical market, diabetes and its related complications continue to be a major medical problem (Guo *et al.*, 2006). The use of medicinal plants for the treatment of diabetes mellitus dates back from the *Ebers papyrus* of about 1550 B.C. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies (Bailey and Day, 1989). Some of the plants which

are being used for the treatment of diabetes have received scientific or medicinal scrutiny and even the WHO expert committee on diabetes recommends that this area warrant further attention (WHO, 1980).

The present study was conducted in two commonly known folkloric medicinal plants namely *Feronia limonia* and *Artocarpus heterophyllus* used by tribal population (Paliyar of Javadhu hills) in treating various illnesses. *Limonia acidissima* Linn, syn. *Feronia limonia* is a moderate sized deciduous tree grown throughout India. The fruits are woody, rough and used as a substitute for bael in diarrhea and dysentery. The fruit contains flavonoids, phytosterols, tannins, carbohydrates, triterpenoids, vitamins and amino acids as its chemical constituents (Intekhab and Aslam, 2009; Kanhiya *et al.*, 2008). Fruit pulp showed significant hepatoprotective and antioxidant activities (Ilango and Chitra, 2009). Many parts of the plant including the barks, root, leaves and fruits are beneficial for humans. An infusion of the mature leaves and bark is used to treat diabetes and Gall stones (Chackrewarthy *et al.*, 2010). Previous studies have indicated that an extract of *Artocarpus heterophyllus* improves the glucose tolerance in normal human subjects and diabetic patients (Femando *et al.*, 1991).

Therefore, the objective of the present study was to evaluate the hypoglycemic activity of *Feronia limonia* and *Artocarpus heterophyllus* extracts in normal and STZ induced diabetic rats. Percent reduction in blood glucose level also monitored and compared with Glibenclamide, a commonly used hypoglycemic agent.

## **MATERIALS AND METHODS**

**Collection of plant material:** *Feronia limonia* Fruit and *Artocarpus heterophyllus* Bark were collected from the Javadhu hills of Tamil Nadu during the month of August-September. The collected materials were identified with the help of Prof. P. Jayaraman, Plant Anatomy Research centre, Chennai. After washing the plant materials thoroughly with water it was dried and coarsely powdered using a cutter mill and stored in an air-tight container.

**Preparation of extract:** A portion of powdered material was defatted with hexane using soxhlet apparatus. The defatted marc was successively extracted with increasing polarity of solvents like hexane, ethyl acetate, methanol and water. The filtrate was evaporated to dryness at 40°C in vacuum. Preliminary phytochemical analysis revealed high content of flavonoids in methanol extract of *Feronia limonia* and ethyl acetate extract of *Artocarpus heterophyllus* compared to other extracts (Reid *et al.*, 2005). So further studies were carried only with these extract samples. Total Flavonoid Content (TFC) was determined by colorimetric method (Chang *et al.*, 2002).

**Selection of animal and induction of diabetes:** Male albino rats weighing 250-300 g were obtained from the VIT University animal house after approval from Institutional Animal Ethical Committee (1333/C/10/CPCSEA). The animals were maintained under standard condition of temperature (23±2°C) and relative humidity (55±10%) with 12 h each of dark and light cycle. Rats were fed with standard pellet and tap water *Ad libitum*. All experimental protocols were prepared and performed based on ethical committee guidelines.

**Chemicals:** Streptozotocin (STZ) used in this experiment was purchased from Himedia Bangalore, Glibenclamide from Sun Pharmaceuticals Limited, Baroda, India. All other chemicals were used of analytical grade.

**Induction of diabetes mellitus:** Diabetes was induced in albino rats as per the method described by Kadnur and Goyal (2005) with slight modification. Briefly, after 18 h fasting rats were made diabetic by single administration of STZ (40 mg kg<sup>-1</sup> b.wt/i.p.) dissolved in 0.1 M citrate buffer (pH 4.5) while the normal rats received citrate buffer as vehicle. After the injection they had free access to feed and 5% sucrose solution in order to overcome hypoglycemic shock. Blood glucose levels were estimated after 3 to 4 days of Streptozotocin injection in overnight fasted rats. Rats having blood glucose level more than 300 mg dL<sup>-1</sup> were selected for further study.

**Experimental design:** Antidiabetic activity was studied in 42 rats (6 normal+36 diabetic rats). They were grouped as follow:

- **Group 1:** Received 1% Tween 80 in water after intraperitoneal injection of citrate buffer (0.1 M)
- **Group 2:** Received 1% Tween 80 in water after intraperitoneal injection of STZ (40 mg kg<sup>-1</sup> body wt.)
- **Group 3 and 4:** Received *Feronia limonia* extract (200 and 400 mg kg<sup>-1</sup> body wt.) administered orally through intragastric tube after 48 h of STZ injection for 30 days
- **Group 5 and 6:** Received *Artocarpus heterophyllus* extract (200 and 400 mg kg<sup>-1</sup> body wt.) administered orally through intragastric tube after 48 h of STZ injection for 30 days
- **Group 7:** Received Glibenclamide (900 µg kg<sup>-1</sup> body wt.) administered orally through intragastric tube after 48 h of STZ injection for 30 days

The body weight and blood glucose level were monitored weekly once for 30 days. Blood samples were obtained by tail vein puncture of both normal and STZ induced diabetic rats and blood glucose level was measured using single touch glucometer. At the end of 31st day all the fasted rats were sacrificed by deep anesthesia. The blood samples were collected by cardiac puncture method and centrifuged at 4000 rpm for 20 min to remove serum from the clot and stored at -20°C. Serum glucose and cholesterol level were estimated using commercially available reagent kits.

**Histopathological study:** Pancreas of control and extract treated animals were isolated for histopathological examination. Isolated pancreas after washing in PBS solution 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, 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

**Hypoglycemic effect of extracts:** Treatment of the rats with Streptozotocin resulted in approximately 2.5-fold increase in blood glucose concentration in comparison to normal control rats. The effect of different concentration of *Feronia limonia* Fruit and *Artocarpus heterophyllus* Bark extracts in lowering the blood glucose level of STZ induced diabetic rats were shown in Table 1. On

Table 1: Effect of *Feronia limonia* and *Artocarpus heterophyllus* extracts on fasting blood glucose

Treatment	Fasting blood glucose level (mg dL <sup>-1</sup> )				
	After STZ	1st week	2nd week	3rd week	4th week
Normal control	93.5±2.34	92.2±6.42	93.7±2.55	94.6±2.48	92.3±3.84
Diabetic control	289.2±4.68**	311.3±5.84	304.4±1.93	306.8±4.63	320.7±2.16
<i>F. limonia</i> extract (200 mg kg <sup>-1</sup> )	304.4 ±3.94**	276.1±3.18* (15.7)	248.2±6.42* (22.3)	243.0±3.89* (24.7)	205.8 ±4.08* (39.0)
<i>F. limonia</i> extract (400 mg kg <sup>-1</sup> )	323.7 ±2.75**	204.5±1.90* (41.3)	196.5±5.44* (42.2)	189.7±9.25* (44.7)	163.4 ±10.42* (54.5)
<i>A. heterophyllus</i> extract (200 mg kg <sup>-1</sup> )	254 ±7.28**	239.2±2.56 (12.5)	227.3 ±2.74 (14.7)	218.6±6.38* (18.8)	228.1 ±7.38* (19.2)
<i>A. heterophyllus</i> extract (400 mg kg <sup>-1</sup> )	326.4±8.13**	283.9 ±8.4* (19.2)	285.4±4.81* (16.7)	276.5±8.14* (20.0)	262.3±8.91* (27.5)
Glibenclamide (900 µg kg <sup>-1</sup> )	318.3±6.32**	192.6 ±4.68* (43.8)	185.7±2.17* (44.5)	168.3 ±7.24* (50.2)	153.8±5.77* (56.5)

Each value represents the Mean±SD (n = 6). The values given in parentheses represent percentage decrease in blood glucose. \*\*p<0.01 compared with the initial level of blood glucose of normal control rats. \*p<0.01 compared with the initial level of blood glucose of the rats in the respective group

administration of plant extract and glibenclamide to the diabetic rats, blood glucose levels were reduced significantly (p<0.01) from day 1 onwards, whereas the blood glucose remained consistently elevated in diabetic control rats throughout the period of study. Percentage reduction in blood glucose level was calculated using the formula:

$$\text{Reduction (\%)} = \frac{1 - (\text{nth day treated} / \text{0th day treated})}{(\text{nth day untreated} / \text{0th day untreated})} \times 100$$

where, n is the day of glucose measurement. Oral administration of methanolic extract of *Feronia limonia* Fruit (200 and 400 mg kg<sup>-1</sup> b.wt.) produced the maximum fall of 39% and 54.5% in blood glucose level of diabetic rats after 4 weeks of treatment. Similarly ethyl acetate extract of *Artocarpus heterophyllus* Bark (200 and 400 mg kg<sup>-1</sup> b.wt.) showed 19.2 and 27.5% reduction in blood glucose level. This is less significant compared to the *F. limonia* whose effect persists even after the treatment schedule of 4 weeks.

**Body weight changes:** The body weight changes in control and diabetic rats were monitored on 1st, 15th and 30th day of experiment. Body weight of diabetic control group was decreased significantly when compared with the normal group, whereas diabetic rats treated with the extract and standard drug regained its original weight after treatment for 30 days. The results were shown in Table 2.

**Serum cholesterol level:** Increased serum cholesterol in diabetic rats when compared with normal control rats is an evidence for hyperlipidemia in diabetic rats. Treatment of diabetic rats with different concentration of extracts resulted in significant fall (p<0.05) in serum cholesterol level compared to the diabetic control rats. *Feronia limonia* (400 mg kg<sup>-1</sup>) treated diabetic rats showed marked reduction in cholesterol level as seen in glibenclamide treated diabetic rats.

Table 2: Effect of *Feronia limonia* and *Artocarpus heterophyllus* extracts on body weight and serum cholesterol level

Treatment	Body weight (g)			Serum cholesterol on 31st day
	0th day	15th day	30th day	
Normal control	312.2±4.6	315.3±1.33	316.6±2.64	92.3±5.23
Diabetic control	326.4±8.16	296.6±1.62	274.0±3.44	114.6±2.81
<i>F. limonia</i> extract (200 mg kg <sup>-1</sup> )	255.6±5.3	241.4±6.3	230.2±8.2	83.7±3.6*
<i>F. limonia</i> extract (400 mg kg <sup>-1</sup> )	235.1±5.2	217.3±4.8	223.5±5.62	76.5±4.7*
<i>A. heterophyllus</i> extract (200 mg kg <sup>-1</sup> )	258±2.84	236.2±3.92	234.5±6.81	91.2±9.22
<i>A. heterophyllus</i> extract (400 mg kg <sup>-1</sup> )	291.3±1.93	263.8±9.21	284.3±3.15	87.6±2.53*
Glibenclamide (900 µg kg <sup>-1</sup> )	264.4±7.41	230.5±8.3	257.4±4.2	76.4±6.3*

Each value represents the Mean±SD (n-6). \*p<0.01 compared with the serum cholesterol of diabetic rats

**Histopathology analysis:** Histopathology study revealed extensive alterations in pancreas of Streptozotocin induced diabetic rats. The pancreas of control rat showing normal islet cells without any congestion/hemorrhages. Diabetic pancreas showing  $\beta$  cell vacuolation and occasional apoptotic cells with vascular congestion and fibrous tissue infiltration in the islet cells of pancreas. Extracts and standard drug (Glibenclamide) treated diabetic pancreas showing initial stages of regenerating islet cells with loss of degenerative features as compared to diabetic pancreas.

## DISCUSSION

In the present study, extracts of *Feronia limonia* fruit and *Artocarpus heterophyllus* Bark at two different concentrations (200 and 400 mg kg<sup>-1</sup> b.wt.) were studied for Antidiabetic activity in STZ induced diabetic rats. Administration of STZ (45 mg kg<sup>-1</sup>) caused rapid destruction of  $\beta$  cells which lead to reduction in insulin release and impaired glucose uptake by peripheral cells and tissues (Aybar *et al.*, 2001). The results of the present study indicated significant glucose lowering effect of *Feronia limonia* Fruit extract when compared to *Artocarpus heterophyllus* Bark extract in STZ induced diabetic rats. Percentage reduction in blood glucose level of extract treated animal increases gradually from day 1 onwards. Maximum fall of 39 and 54.5% were seen in 4th week of *Feronia limonia* extract at a dosage of 200 and 400 mg kg<sup>-1</sup>, respectively. Whereas *Artocarpus heterophyllus* extract treatment results in 19.2 and 27.5% fall in blood glucose level. Glibenclamide being the standard drug for type 2 diabetes acts by stimulating the  $\beta$  cells of pancreas to release insulin (Tian *et al.*, 1998). Standard drug produced the maximum fall of 56.5% in 4th week of treatment.

Literature survey revealed flavonoids and phenols are effective antihyperglycemic agents which can regenerate the damaged  $\beta$  cells in STZ induced diabetic rats (Chakrabarti *et al.*, 2003; Manickam *et al.*, 1997). In the present study, preliminary phytochemical screening of extracts showed the presence of flavonoids, phenols, terpenoids and alkaloids. Antidiabetic activity of extracts may be due to its high content of phenols and flavonoids (Feng *et al.*, 1998). Induction of diabetes by STZ leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins (Chatterjea and Shinde, 2002; Swanston-Flat *et al.*, 1990). These diabetic rats regained its original body weight on treatment with extracts for 30 days. The result from the present study also indicates significant (p<0.05) decrease in serum cholesterol level of diabetic rats treated with extracts compared to diabetic control. Although, *A. heterophyllus* bark extract produced mild glucose lowering effect, serum cholesterol level decreased significantly which may be due to inhibitory effect of the active principles on enzymes of cholesterol biosynthesis (Sharma *et al.*, 2008).

Maximum fall in blood glucose level produced by *Artocarpus heterophyllus* extract was approximately 20 and 24% for lower and higher concentration, respectively. Methanol extract of *Feronia limonia* Fruit showed 27 and 33% reduction in serum cholesterol when compared with the untreated diabetic rats.

## CONCLUSION

In summary, the present study indicated significant dose dependent Antidiabetic effect of *Feronia limonia* Fruit and *Artocarpus heterophyllus* Bark extract in STZ induced diabetic rats. The results suggest that bioactive constituents responsible for improving type 2 diabetic rats need to be isolated and characterized to contribute better therapy for NIDDM.

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