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# ANTI-DIABETIC EFFECT OF *FICUS NERVOSA EXTRACT* IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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**ABSTRACT**

In Indian traditional system of medicine, *Ficus nervosa Heyne ex*. (*Moraceae*) is prescribed for the treatment of diabetes mellitus. In the present study, the antidiabetic effect of petroleum ether extract of *Ficus nervosa* leaves (PEFN) was investigated in streptozotocin (STZ)-induced diabetic rats. Albino wistar rats were rendered diabetic by STZ (55 mg/kg, intraperitoneally). PEFN was orally administered to STZ-induced diabetic rats at 200 and 400 mg/kg, p.o doses for 14 days to determine anti-diabetic activity. The fasting blood sugar levels and serum biochemical analysis in STZ-induced diabetic rats were investigated. Oral administration of PEFN (200 & 400 mg/kg) for 14 days exhibited a significant reduction in serum glucose, total cholesterol, and triglycerides in alloxan diabetic rats. The anti-diabetic activity of the petroleum ether extract of *Ficus nervosa* (PEFN) were similar to those produced by glibenclamide at 600µg/kg (positive control, $p < 0.01$). The results demonstrate that PEFN possesses potent anti-diabetic activity in STZ-induced diabetic rats. These very encouraging results for diabetes control by petroleum ether extract of *Ficus nervosa* make the need for clinical studies in humans evident.

**Keywords:** *Ficus nervosa*, STZ-induced diabetic rats, anti-diabetic, LDL, VLDL, TG, Total cholestrol.

## INTRODUCTION

Diabetes mellitus is a metabolic disorder, characterized by hyperglycemia together with impaired metabolism of glucose and other energy-yielding fuels such as lipids and proteins [1]. The number of people suffering from the diabetes mellitus worldwide is increasing at an alarming rate with a projected 366 million peoples likely to de diabetic by the year 2030 as against 191 million estimated in 2000 [2]. The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. In modern medicine (insulin, sulphonylureas, biguanides and thiazolidinediones), no satisfactory effective therapy is still available to cure the diabetes mellitus. In recent years, there has been renewed interest in plant medicine [3,4] for the treatment against different diseases as herbal drugs are generally out of toxic effect [5,6] reported from research work conducted on experimental model animal.

*Ficus nervosa Heyne ex Roth* belongs to the family *Moraceae.* It is a medium sized evergreen tree in evergreen forests up to 400-1600 m. Aerial roots are absent; leaves are coriacuous, glabrous on both sides, oblanceolate to oblong 8-20 cm long, entire margin, narrowed at base [7,8]. Bark is brown mottled white, wood is white in colour and soft [9]. Madhava Chetty *et al.*, (2008) [8] have given a brief description about the traditional uses of *Ficus nervosa* Heyne ex Roth (Moraceae). In that it has been traditionally used for Diabetes. This includes the leaf as laxative and in the treatment of Diabetes, and rheumatism. In previous study hypoglycemic and hypolipidemic activity of *Ficus nervosa in* alloxan induced diabetes in albino wistar rats was reported. Therefore the present studies to conform the anti-diabetic activity of *Ficus nervosa* in STZ induced diabetic rats model.

## MATERIALS AND METHODS

### Plant Materials

The *Ficus nervosa* leaves were collected in the month of September, 2009 from Thirumala hills in Chittoor district of Andhra Pradesh, India. The leaves were identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi.

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**Preparation of Extract**

The leaves of the plant were collected and dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no.40. Then the powder was extracted with petroleum ether in a Soxhlet extraction apparatus.

**Phytochemical Screening**

The PEFN was tested for the presence of saponins, alkaloids, glycosides, steroids, triterpenoids, flavonoids, tannins, reducing sugars by qualitative and quantitative methods [10].

**Animals Used**

Albino Wistar rats, weighing 150–200 g were used. The selected animals were housed in acrylic cages in standard environmental conditions (20–25 ◦C), fed with standard rodent diet and water *ad libitum*. The experiments on animals were conducted in accordance with the internationally accepted principles for laboratory animal use and the experimental protocols duly approved by the Institutional Ethical Committee. (Reg. No. IAEC/ 930/a/06/ CPCSEA).

**Acute Toxicity Studies**

Acute oral toxicity studies were performed as per OECD-423 guidelines. Male Wister mice were used for the study. The animals were divided into six groups containing six animals in each group. The extract was administered orally at the doses from 200- 2000mg/kg. There were no signs of toxicity and mortality was observed up to 2000mg/kg [11].

**Experimental induction of diabetes**

The animals were fasted overnight and diabetes was induced by a single intraperitoenal injection of a freshly prepared solution of streptozotocin (STZ) (55 mg/kg body weight) in 0.1 M cold citrate buffer (pH 4.5) [12]. The animals were allowed to drink 1% glucose solution overnight to overcome the drug- induced hypoglycemia. Control rats were injected with citrate buffer alone. The animals were considered as diabetic, if their blood glucose values were above 250 mg/dl on the third day after STZ injection. The treatment was started on the fourth day after STZ injection and this was considered as first day of treatment. The treatment was continued for 14 days.

**Experimental design**

The rats were divided into four groups comprising of six animals in each group as follows:

Group I. Control rats receiving 0.1 M citrate buffer (pH 4.5).

Group II. Diabetic control and rats received only vehicle (2 ml/kg p.o) 2% v/v Tween 80.

Group III. Diabetic rats received Petroleum ether extract of *Ficus nervosa* (200 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group IV. Diabetic rats received Petroleum ether extract of *Ficus nervosa* (400 mg/kg/day p.o) suspended in 2% v/v Tween 80.

Group V. Diabetic rats treated with glibenclamide (600 µg/kg b.w/day) suspended in 2% v/v Tween 80 orally for 14 days [13].

**Testing of fasting blood glucose level**

Fasting blood glucose levels were measured on 0, 3, 7, and 14 days of treatment of Petroleum ether extract of *Ficus nervosa leaves* supplement from the animals of all these groups. Blood was collected from tip of the tail vein and fasting blood glucose level was measured using single touch glucometer [14]. The results were expressed in terms of milligram per deciliter of blood.

At the end of the experimental period, all the animals were sacrificed under light ether anesthesia. The guide line of our institutional ethical committee for this purpose was followed strictly. The rats were sacrificed by decapitation and blood was collected with anti-coagulant and the serum was used for the estimation of total cholesterol and triglycerides. Total cholesterol was estimated by the method of Parekh and Jung (1970) [15]. Triglycerides were estimated by the method of Rice (1970) [16].

**Histopathological study of pancreas**

Pancreas were isolated and preserved in 10% formalin. Histopathological observation of the tissue was carried out at the Sri Venkateswara University, Pathology Laboratory, Tirupati, Andhra Pradesh -517 502.

**Statistical Analysis**

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnet's test p values less than 0.05 were considered as significance.

**RESULTS**

**Effect of PEFN on fasting blood glucose in STZ-induced diabetic rats**

The effect of repeated oral administration of PEFN on blood glucose levels in STZ-diabetic rats is presented in Table 1. PEFN, administered at doses of 200 & 400 mg/kg to STZ-treated diabetic rats caused significant ($P < 0.01$) reduction of blood glucose levels which was related to dose and duration of treatment. Maximum reduction was observed on day 14. PEFN 400 mg/kg exhibited maximum glucose lowering effect in diabetic rats. Glibenclamide exhibited significant reduction in blood glucose levels at the end of the study when compared to diabetic control.

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**Effect of PEFN on serum lipids in STZ-induced diabetic rats**

PEFN showed a dose related significant ($P < 0.01$) reduction in triglycerides compared to pretreatment levels (Table 2). PEFN at the doses of 200 and 400 mg/kg was dose dependently reduced the Total cholesterol, LDL, VLDL, TG levels than diabetic control rats.

**Table 1: Effect of Ficus nervosa on fasting blood glucose levels of STZ- induced diabetic rats**

| Groups (n=6) | Fasting Blood Glucose Levels | | | |
|---|---|---|---|---|
| | 0th Day | 5th Day | 10th Day | 15th Day |
| Group-I (Normal Control) | 95 ± 1.15 | 95.66 ± 1.14** | 95.16 ± 0.79** | 95.5 ± 0.76** |
| Group-II (Diabetic Control) | 279.5 ± 2.47 | 272.33 ± 2.19 | 282.33 ± .04 | 293.66 ± 2.96 |
| Group-III (PEFN-200mg/kg) | 276.5 ± 2.75[a] | 231.5 ± 1.97**[a] | 167.66 ± 2.46**[a] | 123.16 ± .41**[a] |
| Group-IV (PEFN-400mg/kg) | 282.16 ± 3.89[a] | 229 ± 3.44**[a] | 161.83±1.04**[a] | 120.5 ± 1.28**[a] |
| Group-V (Glibenclamide - 600 µg/kg) | 281.5 ± 3.05[b] | 206.33 ± 2.24**[b] | 138.16 ± 3.56**[b] | 100 ± 0.66**[b] |

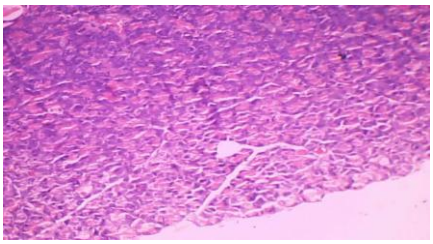
Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. diabetic + PEFN - 200 & 400 treated rats vs. diabetic control rats. **b**. diabetic + Glibenclamide treated rats vs. diabetic control rats.

**Table 2: Effect of *Ficus nervosa* on Total lipid profile of STZ- induced diabetic rats**

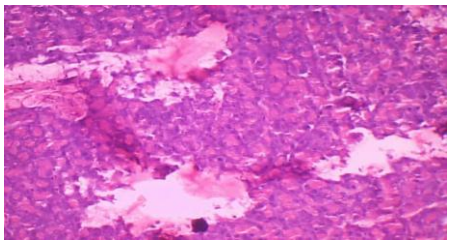
| Groups (n=6) | Biochemical Parameters | | | | |
|---|---|---|---|---|---|
| | TG (mg/dl) | TC (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
| Group-I (Normal Control) | 80.72±0.43**[a] | 90.86±0.17**[a] | 34.39±0.50**[a] | 33.14±0.63**[a] | 15.01±0.90**[a] |
| Group-II (Diabetic Control) | 137.57±1.10 | 152.72±5.89 | 15.87±4.19 | 75.52±4.19 | 48.2±5.12 |
| Group-III (PEFN-200mg/kg) | 112.45±0.59*[b] | 97.58±0.44**[b] | 25.39±0.25**[b] | 38.65±0.59**[b] | 28.52±0.44**[b] |
| Group-VI (PEFN-400 mg/kg) | 95.00±0.47**[b] | 89.04±0.49**[b] | 27.93±0.39**[b] | 35.07±0.35**[b] | 24.07±0.47**[b] |
| Group-V (Glibenclamide - 600 µg/kg) | 84.14±0.61**[c] | 82.00±0.49**[c] | 28.71±0.34**[c] | 32.17±0.25**[c] | 20.52±0.58**[c] |

Values are given as mean ± SEM for groups of six animals in each group. Values are statistically significant at *p<0.05 and **p<0.01 and ns-non significant. Significance compared within the groups as follows: **a**. Normal control rats vs. diabetic control rats. **b**. diabetic + PEFN - 200 & 400 treated rats compared with diabetic control rats. **c**. diabetic + Glibenclamide treated rats vs. diabetic control rats.

**HISTOPATHOLOGICAL STUDIES**



**Group-I (Normal Control)**



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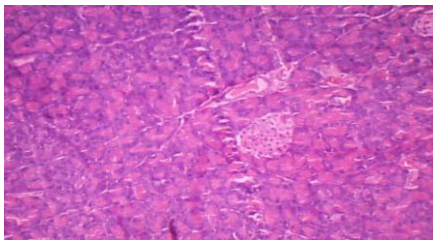
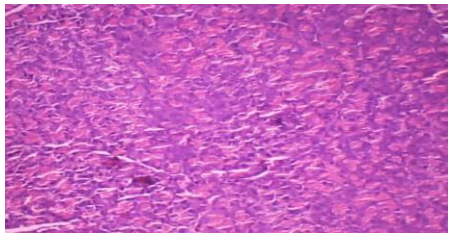
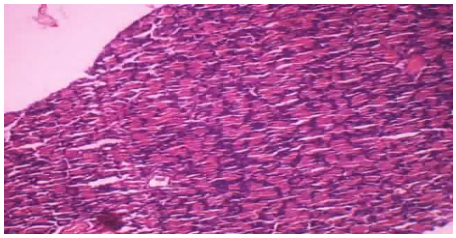


**Group-III (PEFN-200mg/kg)**



**Group-IV (PEFN-400mg/kg)**



**Group-V (Glibenclamide - 600 µg/kg)**

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**Discussion and conclusion**

The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs [17]. The aim of the present study was to evaluate the anti-diabetic effects of *Ficus nervosa* in STZ-induced diabetic rats. The experimental diabetic model used in this study was type 2 since low dose of STZ (55 mg/kg bw) destroyed some population of pancreatic beta cells [18]. There were residual beta cells which secreted insufficient insulin causing type 2 diabetic model [19].

The mechanism by which streptozotocin STZ, a highly cytotoxic agent of pancreatic β-cells [20], induces diabetes by damaging the cells that causes reduction in insulin. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of *Ficus nervosa* . The reduced glucose levels suggested that *Ficus nervosa* might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis [21,22], or by absorption of glucose into the muscle and adipose tissues [23] through the stimulation of a regeneration process and revitalisation of the remaining beta cells [24-26].

The possible mechanism by which PEFN mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets, as was evident by the significant increase in the level of insulin in the extract treated animals. In this context, a number of other plants have been reported to have antihyperglycemic activity with a stimulatory effect on insulin release [20,27]. Since the extract produced highly significant antihyperglycemic effect even in streptozotocin-induced diabetic rats in which most of the β- cells are damaged, it is likely that PEFN might have extra pancreatic mechanism of action. The other effects such as increase in the levels of total cholesterol, triglycerides and LDL-cholesterol and decrease in HDL-cholesterol of eremanthin in STZ-treated animals could be secondary to a partially restored beta-cell function with increased insulin levels. From the results of the present study, it may be suggested that the mechanism of action of PEFN may be similar to glibenclamide action.

In diabetes, hyperglycemia is accompanied with dyslipidemia [28,29], i.e. characterized by increase in TC, LDL, VLDL, TG and fall in HDL. Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart diseases which are the secondary complications of diabetes [30]. This altered serum lipid profile was reversed towards normal after treatment with the PEFN. PEFN exhibited hypocholesterolemic and hypotriglyceridemic effects, while increased the levels of HDL in streptozotocin-induced diabetic rats. However, PEFN was found to be more effective in reducing the levels of TG and LDL as compared to its effect on TC. The elevated atherogenic index, i.e. TC/HDL ratio, which is a useful determinant of cardiovascular risk [31], was also shifted towards normal after PEFN treatment. Thus, it is reasonable to conclude that PEFN could modulate blood lipid abnormalities.

Summarizing, it could be proofed that the traditional use of *Ficus nervosa* as a hypoglycaemic agent is justified and that extracts from this plant show a dose-dependent activity which is comparable to the standard hypoglycaemic drug glibenclamide. Further studies to isolate, identify and characterize the active principle(s) are in the progress.

**References**

1. Scheen JA. Drug treatment of non-insulin dependent diabetes mellitus in the 1990s. Achievements and future development. *Drugs,* 54, 1997, 355–368.
2. SG Wild A, Roglic R, Green H, King. Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030, *Diabetes Care,* 27, 2004, 1047–1054.
3. Prince PS, Menon VP, Pari L. Hypoglycemic activity of *Syzigium cuminii* seeds: effect on lipid peroxidation in alloxan diabetes rats. *Journal of Ethnopharmacology,* 61, 1998, 1–7.
4. Ladeji O, Omekarah I, Solomon M. Hypoglycemic properties of aqueous bark extract of *Ceiba pentandra* in streptozotocin-induced diabetic rats. *Journal of Ethnopharmacology,* 84, 2003, 139–142.
5. Geetha BS. Biju CM, Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from *Ficus bengalensis* (Linn.). *Indian Journal of Pharmacology*,38, 1994, 220–222.
6. Rao BK, Sudarshan PR, Rajasekhar MD, Nagaraju N, Rao CA. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. *Journal of Ethnopharmacology*, 85, 2003, 169–172.
7. Anonymous 1- www.efloras.org, Flora of China, 5, 46.
8. Madhava Chetty K, Sivaji K, Tulasi Rao K, Flowering plants of Chittoor District, Andhra Pradesh, India, Students Offset Printers, Tirupathi, 333-334.
9. Gamble JS, Flora of the Presidency of Madras, Botanical Survey of India, Calcutta, 1967, 2nd reprinted ed., 954.

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10. Gokhale SB, Kokate CK, Purohit, Pharmacognosy, Nirali Prakashan Publishers, Pune, Nineteenth Edition, 2004, 8.1-8.23.
11. OECD 2002. Acute oral toxicity. Acute oral toxic class method guideline 423 adopted 23.03.1996. In: Eleventh Addendum to the, OECD, guidelines for the testing of chemicals organisation for economical co-operation and development, Paris, June, 2000.
12. Rakieten N, Rakieten ML, Nadkarni MV. Studies on the diabetogenic action of streptozotocin (NSC-37917). *Cancer Chemo Therapy Reports* 29, 1963, 91–98.
13. Pari L, Uma Maheswari J. Antihyperglycemic activity of Musa sapientum flowers: effect on lipid peroxidation in alloxan diabetic rats. *Journal of Ethnopharmacology*, 14, 2000, 136–138.
14. Atkin SH, Dasmahapatra A, Jaker MA, Chorost MI, Reddy S. Fingerstick glucose determination in shock. *Annual International Medicine*, 114, 1991, 1020–1024.
15. Parekh AC, Jung DH. Cholesterol determination with ferric acetate and sulphuric acid-ferrous sulphate reagents. *Analytical Chemistry*, 42, 1970, 1423–1427.
16. Rice EW. Triglycerides in serum. In: Roedrick, P., McDonal, R.P. (Eds.), Standard Methods in Clinical Chemistry. Academic Press, New York, 1970, 215.
17. Grover JK, Vats V, Rathi SS. Antihyperglycemic effect of Eugenia jambolana and Tinospora cordifolia in experimental diabetes and their effects on key metabolic enzymes involved in carbohydrate metabolism, *J. Ethnopharmacol*. 73, 2000, 461–470.
18. Aybar M, Riera ANS, Grau A, Sanchez SS. Hypoglycemic effect of the water extract of Smallanthus soncifolius (yacon) leaves in normal and diabetic rats, *J. Ethnopharmacol*. 74, (2002) 125–132.
19. Gomes A, Vedasiromoni JR, Das M, Sharma RM, Ganguly DK. Antihyperglycemic effect of black tea (Camellia sinensis) in rat, *J. Ethnopharmacol*. 27, 2001, 243–275.
20. Esmaeili MA, Yazdanparas R. Hypoglycemic effect of *Teucrium polium*: studies with rat pancreatic islets. *Journal of Ethnopharmacology*, 95, 2004, 27–30.
21. Ali L, Azad Khan AK, Mamun MIR, Mosihuzzaman M, Nahar N, Alan MNE, Rokeya B. Studies on the hypoglycemic effects of fruits pulp, seed and whole plant of Momordica charantia on normaland diabetic model rats, *Planta Med*. 59, 1993, 408–412.
22. Gray AM, Abdel-Wahab YHA, Flatt PR. The traditional plant treatment, Sabucus nigra (Elder), exhibits insulin-like and insulin releasing actions in vitro, *J. Nutr.* 130, 2000, 15–20.
23. Kamanyi A, Dajmen D, Nkeh B. Hypoglycemic properties of the aqueous root extract of Morinda lucida (Rubiacea) study in the mouse, *Phytother res.* 8, 1994, 369–371.
24. Rokeya B, Nahar N, Ali L, Hassan Z, Alam MNE, Choudhury SN, Azad Khan AK, Mossihuzzaman M. Effects of five medicinal plants on blood glucose levels in non-diabetic and diabetic model rats, *Diabetes Res.* 34,1999, 219– 228.
25. Shanmugasundaram ERB, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocindiabetes rats given Gymnema sylvetre leaf extracts, *J. Ethnopharmacol*. 30, 1990, 265–279.
26. Bolkent S, Yamardag R, Tabakogluoguz A, Sacaon OO. Effects of chord (Beta vulgaris L.Var.cicla) extract on pancreatic β-cells in Streotozotocin-diabetic rats: a morphologic and biochemical study, J. *Ethnopharmacol*. 73, 2000, 251–259.
27. Sharma SB, Nasir A, Prabhu KM, Murthy PS. Antihyperglycemic effect of the fruit-pulp of *Eugenia jambolana* in experimental diabetes mellitus. Journal of Ethnopharmacology 104, 2006, 367–373.
28. Bierman EL, Amaral JAP, Belknap BH. Hyperlipidemia and diabetes mellitus. *Diabetes*, 15, 1966, 675–679.
29. Garber AJ. Attenuating CV risk factors in patients with diabetes: clinical evidence to clinical practice. *Diabetes, Obesity and Metabolism*, 4, 2002, S5–S12.
30. Ananthan R, Latha M, Ramkumar K, Pari L, Baskar C, Bai V. Effect of Gymnema montanum leaves on serum and tissue lipids in alloxan diabetic rats. *Experimental Diabetes Research*, 4, 2003,183–189.
31. Grover SA, Levington C, Paquet S. Identifying adults at low risk for significant hyperlipidemia: a validated clinical index. *Journal of Clinical Epidemiology*, 52, 1999, 49–55.