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EVALUATION OF ANTIDIABETIC ACTIVITY OF NYCTANTHES ARBOR-TRISTIS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

Background: *Nyctanthes arbor-tristis* Linn are used extensively in Ayurvedic medicine for the treament of diabetes. The present study was carried out to evaluate the antidiabetic action of ethanolic extract of *Nyctanthes arbor-tristis* in Streptozotocin induced diabetic albino rats. **Methods**: To look for the antidiabetic effect, the albino rats were divided into 5 groups, each consisting of 6 animals. Diabetes was induced by a single i.p. injection of Streptozotocin at a dose of 65 mg/kg body weight. Standard drug, Glibenclamide and ethanolic extract of *Nyctanthes arbor-tristis* at doses 250 mg/kg and 500 mg/kg body weight was fed to the rats and it was continued till the end of the study. The blood glucose levels were estimated on day 0, 7, 14 and 21 day. The standard drug and the extract were fed from day 4 onwards. **Results**: The antidiabetic property of the extract has shown increasing trend with increase in dose and there was a gradual decrease in blood glucose levels with increased period of exposure to the test drug. Conclusions: Results obtained in this study substantiate the anti-diabetic activity of *Nyctanthes arbor-tristis*.

KEYWORDS: Antidiabetic, *Nyctanthes arbor-tristis*, Streptozotocin.

INTRODUCTION

Diabetes is one of the major chronic non-communicable metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels.[1] Diabetes is classified on the basis of the pathogenic process that leads to hyperglycemia, as opposed to earlier criteria such as age of onset or type of therapy. The two broad categories of diabetes mellitus are designated as type 1 and type 2. Type 1 diabetes mellitus results from autoimmune beta cell destruction, which leads to insulin deficiency. Type 2 diabetes mellitus is a heterogeneous group of disorders characterized by variable degrees of insulin resistance, impaired insulin secretion and increased glucose production. Although type 1 diabetes mellitus most commonly develops before the age of 30, autoimmune beta cell destruction can develop at any age. Type 2 diabetes mellitus develops more rapidly with increasing age, but it also occurs in children, particularly in obese individuals.[2] The global prevalence of diabetes is estimated to increase from 4% in 1995 to 5.4% by the year 2025 and the number of people with DM are set to rise from an estimate of 150 million in 2008 to 220 million in 2010 and 300 million in 2025.[3,4] The countries with the largest number of diabetic people are and will be in the year 2025, India, China and United States.[3] 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.[5] Despite the availability of insulin and many oral hypoglycemic drugs diabetes mellitus still remains a major health concern for humans.

Therefore new therapeutic approaches are needed to treat diabetes more efficiently. There is a growing interest in focusing on the beneficial role of "alternative therapeutics" in the treatment of diabetes.[6]

Nyctanthes arbor-tristis Linn is native to India, distributed widely in sub-Himalayan regions and southward to Godavari (Das S *et al.*, 2010). It is also distributed in Bangladesh, Indo-Pak subcontinent and South-East Asia (Khatune NA *et al.*, 2003), tropical and sub-tropical South East Asia. It grows in Indo-Malayan region and distributed across Terai tracts as well as Burma and Ceylon. It tolerates moderate shade and is often found as undergrowth in dry deciduous forests

The leaves of *Nyctanthes arbor-tristis* Linn are used extensively in Ayurvedic medicine for the treament of

various diseases such as sciatica, chronic fever, rheumatism and internal worm infections and as a laxative, diaphoretic and diuretic. Leaves are used in cough. Leaf juice is mixed in honey and given thrice daily for the treatment of cough. Paste of leaves is given with honey for the treatment of fever, high blood pressure and diabetes. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic. Leaves are also used in the enlargement of spleen. The leaf juice is used to treat loss of appetite, piles, liver disorders, biliary disorders, intestinal worms, chronic fever, obstinate sciatica, rheumatism and fever with rigors. The extracted juice of leaves acts as a cholagogue, laxative and mild bitter tonic. It is given with little sugar to children as a remedy for intestinal ailments. In several cases, it has been found to act efficaciously for malaria fever. The decoction of leaves is extensively used by Ayurvedic physicians for the treatment of arthritis, obstinate sciatica, malaria, intestinal worms and as a tonic, cholagogue and laxative. The expressed juice of leaves (10 ml BD × 5 days) is a native remedy for intermittent fever.

MATERIALS AND METHODS

Ethical review

The experimental protocol has been approved by institutional animal ethical committee, JKKMMRF College of Pharmacy, B.Komarapalayam, Namakkal Proposal number: JKKMMRFCP/IAEC/2016/001.

Drugs and chemicals used in the study

Streptozotocin (purchased from Sigma Chemical Co., St. Louis, MO, USA), Carboxy methyl cellulose, sodium salt, fructose, phosphotungstic acid, 1,1',3,3' tetramethoxy propane, butylated hydroxy toluene, xylenol orange, dithionitro bis benzoic acid, ascorbic acid, 2,2' dipyridyl, p-phenylene diamine hydrochloride, sodium azide, isopropanol, sodium meta periodate and triethanolamine (from S.D. Fine Chemicals, Mumbai, India) Hesperidin (purchased from Himedia Laboratories Pvt, Ltd, Mumbai) Blood glucose and ELISA kits (purchased from Agappe diagnostics, Kerala, India).

Plant material

Nyctanthes arbor-tristis Linn is native to India, distributed widely in sub-Himalayan regions and southward to Godavari (Das S et al., 2010). The bark were shade dried and then ground to a coarse powder using a mixer grinder. Powder was tightly packed in Soxhlet apparatus and extracted employing ethanol as solvent for 5 days at a temperature of 40-60°C using a heating mantle. The extract was filtered using Whatman filter paper.

Experimental animals used in the study

The study was carried out in healthy albino rats of Sprague Dawley variety of either sex weighing between 150-250 gm. The animals were fed on rat chaws diet and water ad libitum during the experiment. Animals were maintained under controlled condition with 12 hour light and 12 hour dark cycles at a temperature of 24 C and humidity of 55±5%. Before conducting the experiment all the animals were acclimatized to laboratory condition for 7 days. The animals were housed in separate polypropylene cages inside a well ventilated room and their bedding changed from time to time.

Acute toxicity study

The study carried out acute toxicity studies on the water soluble fraction of ethanolic extract of different parts of *Nyctanthes arbor-tristis* Linn at doses of 400 mg/kg to 2000 mg/kg i.p. by staircase method. (Das *et al.*, 2008).

Induction of diabetes in rats

The animals were acclimatized for one week before initiation of the experiment. After overnight fasting, diabetes was induced by intraperitoneal injection of streptozotocin (STZ), 15 min after the administration of 110mg/kg body weight of nicotinamide STZ was freshly dissolved in 0.1 m sodium citrate buffer, pH 4.5 at a dose of 65 mg/kg body weight. After 48 hours of STZ administration rats with moderate diabetes having hyperglycemia (>250mg/dL) were used in this study. The treatment was started on the third day after the STZ injection and this was considered the first day of treatment. The treatment was continued for 21 days (Pari *et al.*, 2006).

Experimental Design

The rats were divided into five groups comprising 6 animals in each group as follows

Group 1: Control rats given only Carboxy Methyl Cellulose (CMC).

Group 2: Diabetic controls (STZ 65 mg/kg + 110 mg/kg Nicotinamide)

Group 3: Diabetic rats treated with Glibenclamide (10 mg/kg p.o) for 21days

Group 4: Diabetic rats treated with Ethanolic Extract of *Nyctanthes arbor-Tristis* Linn Bark (250 mg/kg body weight p.o.) Suspended in CMC for 21 days.

Group 5: Diabetic rats treated with Ethanolic Extract of *Nyctanthes arbor-Tristis* Linn Bark (500 mg/kg body weight p.o.) Suspended in CMC for 21 days.

After 21 days of treatment period the fasted rats were sacrificed by cervical decapitation and the blood was collected in tubes containing potassium oxalate and sodium fluoride as anticoagulant for estimation of fasting plasma glucose. Plasma and serum were separated by centrifugation. After centrifugation at 2,000 rpm for 10 min, the clear supernatant was used for the analysis of various biochemical parameters. The liver was excised and rinsed in ice-cold saline and kept in formalin solution. Tissues were cut into small pieces and homogenized with a Potter-Elvehjem tight-fitting glass-Teflon homogenizer in Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and the supernatant was used for various measurements.

Statistical analysis

All the values are expressed as mean ± S.E.M for groups of six animals each. Analyzed by one way ANOVA and compared by using Tukey-Kramer multiple comparison test using Instat v.2.02 software (GraphPad Software Inc). The values are statistically significant at three levels, ***p<0.001, **p<0.01, *p<0.05. But ns of p>0.05.

RESULTS

On day 0 i.e. the day when streptozotocin was injected, prior to its administration the blood glucose levels were evaluated in all the rats.

Effect of EENA on Oral Glucose Tolerance Test in Rats Table 1 Oral Glucose Tolerance Test

Groups	Fasting	30 min	60 min	90 min	120 min
Control	82.5 ± 3.16	115.84 ± 2.13	112.2 ± 3.84	109.2 ± 2.44	106.45 ± 2.17
Diabetic + Glibenclamide (10mg / kg)	77.4 ± 1.77	96.61 ± 2.10 *	94.2 ± 1.95 *	90.82± 1.65 *	89.35 ± 1.03 *
Diabetic + EENA (250 mg/kg)	78.3 ± 1.65	107.2 ± 0.85 *	105.3 ± 1.19 *	100.3 ± 1.95 *	98.4 ± 1.65 *
Diabetic + EENA (500 mg/kg)	76.35 ± 0.825	102.25 ± 1.25 *	98.35 ± 1.25 *	96.9 ± 2.32 *	94.9 ± 1.795 *

Values are given as mean ± Standard error mean (S.E.M) for groups of six animals each. Values are statistically significant at * P<0.05, **P<0.01, ***P<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

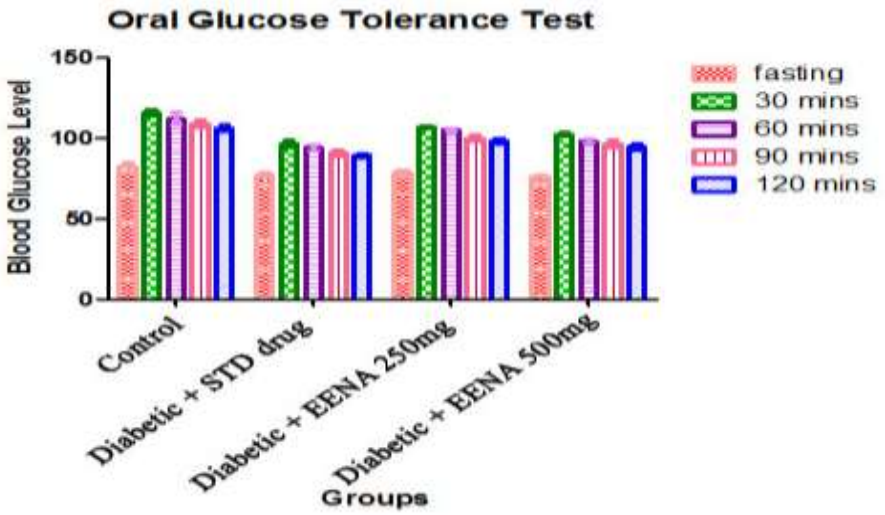

Figure: 1 Effect of EENA on Oral Glucose Tolerance Test in Rats.

Table 2: Body Weight Changes of EENA on Control and Experimental Groups of Rats.

Treatment	Body weight (g) Initial	Body weight (g) Final
Control	179.1±3.3	194.9±3.9
Diabetic Control	181.1±3.1	165.3±3.3
Diabetic + Standard drug (10 mg / kg)	175.1±5.6**	192.1±5.04**
Diabetic + Extract 250 mg / kg (EENA 250 mg / kg)	179.1±3.6*	183.1±3.4*
Diabetic + Extract 500 mg / kg (EENA 500 mg / kg)	175.1±3.0**	189.85±3.9**

Values are given as mean ± Standard error mean (S.E.M) for groups of six animals each. Values are statistically significant at * P<0.05, **P<0.01, ***P<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

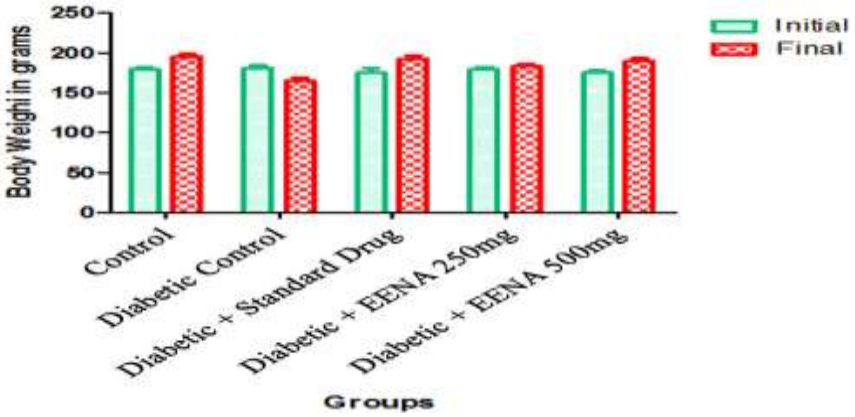

Figure: 2 Effect of EENA on Body Weight of Control and Experimental Groups of Rats.

Table: 3 Effect of EENA on Blood Glucose of Control and Experimental Groups of Rats.

Groups	Blood Glucose Level (mg / dl)			
	0 Day	7th Day	14th day	21st day
Normal control	92.16 ± 3.25	96.23 ± 2.73	94.9 ± 4.65	97.1 ± 1.81
Diabetic control	365.93±1.10***	374.76 ± 4.38***	390.3 ± 4.25***	405.2 ± 7.95***
Diabetic + Glibenclamide 10 mg / kg	305.1 ± 4.59*	250.5 ± 4.26***	204.5 ± 5.24***	139.1 ± 9.33***
Diabetic + EENA 250 mg / kg	306.7 ± 4.02*	265.5 ± 12.26***	217.7 ± 9.67***	178.6 ± 6.41***
Diabetic + EENA 500 mg / kg	307.93 ± 3.58*	253.5 ± 7.54***	202.5 ± 4.86***	141.9 ± 3.42***

Values are given as mean ± Standard error mean (S.E.M) for groups of six animals each. Values are statistically significant at * P<0.05, **P<0.01, ***P<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

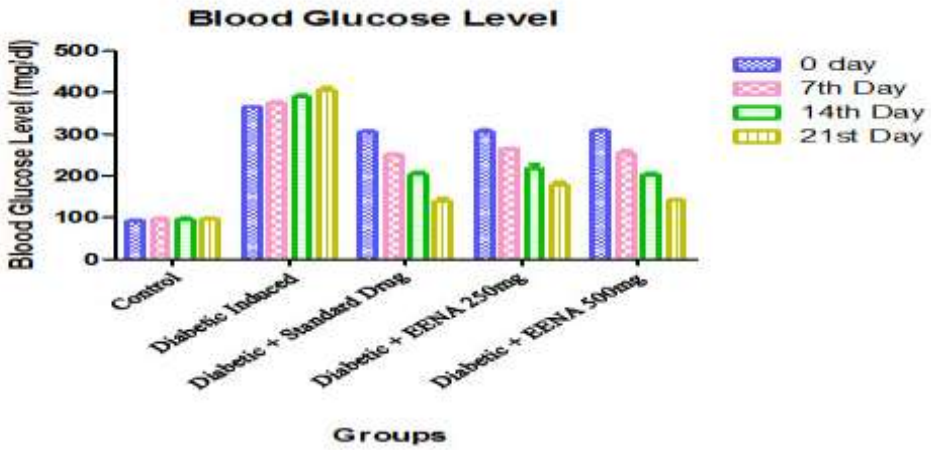

Figure 3: Effect of EENA on Blood Glucose of Control and Experimental Groups of Rats.

Table 4: Effect of EENA on Serum Insulin of Control and Experimental Groups of Rats.

Group	Serum Insulin	HbA_1C
	Final	Final
Control	29.17±0.46	1.92 ± 0.17
Diabetic Induced	19.96±0.59**	5.80 ± 0.37**
Diabetic + Glibenclamide 10 mg / kg	27.12±0.47 *	2.10 ± 0.15**
Diabetic + EENA 250 mg / kg	23.32±0.68 *	4.30 ± 0.38 *
Diabetic + EENA 500 mg / kg	26.61±0.68 *	3.30 ± 0.44 **

Values are given as mean ± Standard error mean (S.E.M) for groups of six animals each. Values are statistically significant at * P<0.05, **P<0.01, ***P<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

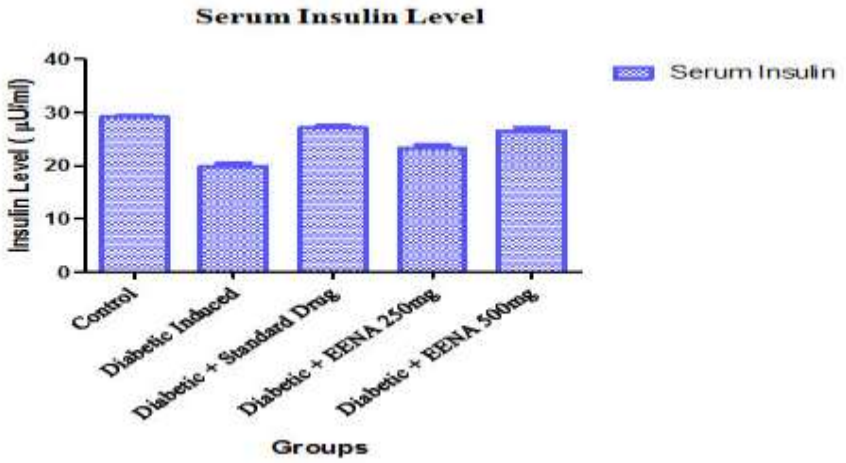

Figure 4.1: Effect of EENA on Serum Insulin of Control and Experimental Groups of Rats.

Serum Insulin Level

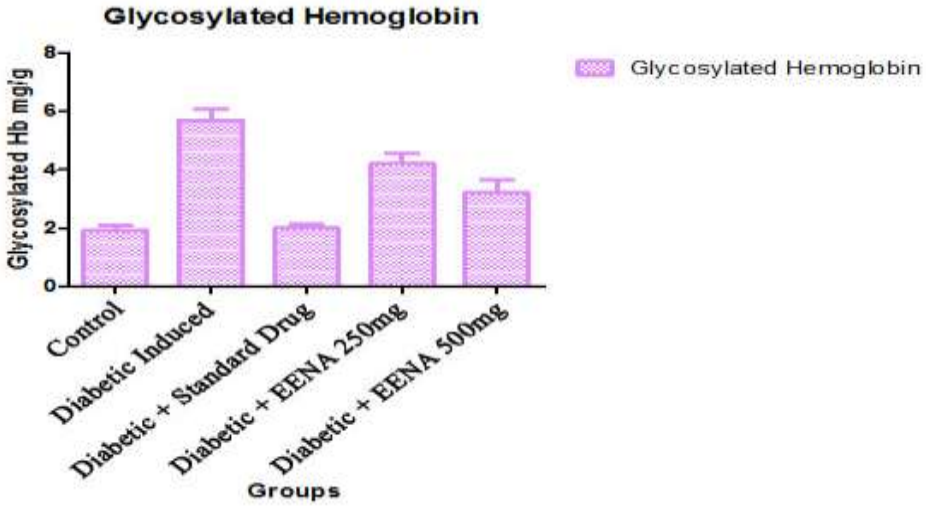

Figure 4.2: Effect of EENA on Glycosylated Hemoglobin of Control and Experimental Groups of Rats.

Table 5: Effect of EENA on Total Cholesterol, Triglycerides, HDL Cholesterol, LDL Cholesterol, VLDL Cholesterol of Control and Experimental Groups of Rats.

Groups	Total Cholesterol (mg / dl)	Triglycerides (mg / dl)	HDL cholesterol (mg / dl)	LDL cholesterol (mg / dl)	VLDL cholesterol (mg / dl)
Control	132.1±3.1	82.1±1.68	48.71±1.86	40.9±1.56	19.1±1.14
Diabetic control	255.4±2.73***	206.7±4.78***	28.38±1.854**	150.1±1.64***	34.3±1.71***
Diabetic + Standard Drug (10 mg/kg)	160.88±1.27**	87.7±2.274**	52.9±2.358**	46.1±1.54**	20.5±1.28**
Diabetic + Ethanol Extract (250 g/kg)	180.1±2.31*	119.5±2.239*	38.27±1.77*	83.1±2.24*	29.7±1.07*
Diabetic + Ethanol Extract (500 mg/kg)	165.76±2.13**	95.9±1.97**	43.55±2.27**	51.1±1.14**	24.3±2.227**

Values are given as mean ± Standard error mean (S.E.M) for groups of six animals each. Values are statistically significant at * P<0.05, **P<0.01, ***P<0.001. Group II compared with group I and Groups III, IV & V were compared with group II.

DISCUSSION

Due to the nature and complexity of diabetes and the lack of an effective cure, traditional herbal medicine or alternative medicine as it is known in the scientific world today has been explored for potential ways to control, manage and cure diabetes.[7] Medicinal plants are a rich source of natural products and these have been used for the treatment of diabetes all around the world with less known scientific basis of their function.[8]

The STZ induced diabetic rat is one of the animal models of human diabetes mellitus. Diabetes arises from irreversible destruction of pancreatic β cells, causing reduction of insulin secretion.

The observed increased blood glucose level in the study is in agreement with reports by several workers that STZ induced diabetes mellitus leads to increased blood glucose. It has been reported that STZ at lower doses produce partial destruction of pancreatic β cells with permanent diabetes condition and there is possibility of many surviving β cells. Since a low dose of STZ (50mg/kg body wt. i.p.) was chosen for this study there might have been many surviving β cells, capable of undergoing regeneration.[9]

Glibenclamide, a standard hypoglycemic agent was taken for comparison of the glucose lowering effectiveness of the ethanolic extract of *Nyctanthes arbor-tristis*.

Albino rats have been used for experimental models of hyperglycemia. They are the commonest laboratory animals suitable for experimental work because of their small size, greater sensitivity to most drugs, easy breeding and resemblance to human nutritionally.[10]

The study shows that the ethanolic extract of *Nyctanthes arbor-tristis* has antidiabetic potential is equal to standard drug, glibenclamide.

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