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Anti Diabetic activity of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn.*

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ABSTRACT

The stem bark of *Nyctanthes arbor-tristis linn* possesses several bioactivities and is used in traditional medicinal systems. However, its antidiabetic activity has not been scientifically investigated so far. The aim of this study was to investigate the antidiabetic activity of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn*. This was tested in streptozotocin (STZ) - nicotinamide induced diabetic rats using oral administration of ethanol extract (EENA). In diabetic rats, the ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* significantly lowered the blood glucose level in a dose-dependent manner. In glucose tolerance test, the extracts at the doses of 250 and 500 markedly reduced the external glucose load. The antidiabetic activity of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* is comparable to that of diabetic control animals. It is concluded that ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* possess safe and strong antidiabetic activity.

Keywords: *Nyctanthes arbor-tristis linn,* Streptozotocin, Nicotinamide, Glibenclamide, Anti-diabetic activity.

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INTRODUCTION

Nyctanthes arbor-tristis Linn. commonly known as Harsinghar or Night Jasmine is one of the well known medicinal plants. Different parts of *Nyctanthes arbor-tristis* are known to possess various ailments by rural mainly tribal people of India (Orissa and Bihar) along with its use in Ayurveda, Sidha and Unani systems of medicines. Juice of the leaves is used as digestives, antidote to reptile venoms, mild bitter tonic, laxative, diaphoretic and diuretic [1-3]. Leaves are also used in the enlargement of spleen.

Traditionally the powdered stem bark is given in rheumatic joint pain, in treatment of malaria and also used as an expectorant [2]. The claimed traditional medicinal uses have been proved on scientific basis using *in-vitro* and *in-vivo* experiments. The plant have been screened for antihistaminic activity, CNS activities (viz. hypnotic, tranquillizing, local anesthetics), analgesic, anti-inflammatory, antipyretic, antiulcer, amoebicidal, anthelmintic, antitrypanosomal to antidepressant, antiviral and immunomodulatory [4]. Leaves extracts was found to have antimicrobial activity [5] but no report is available on the antidiabetic activity on the stem bark part so the present study is aimed at the screening of the antidiabetic activity in the stem bark extracts of the plant *Nyctanthes arbor-tristis Linn*.

MATERIALS AND METHODS

Plant Material

After proper identification of the Taxonomists in the Botanical Survey of India, Coimbatore, Tamil Nadu, the stem bark of the plant *Nyctanthes arbor-tristis linn* was collected from the surrounding areas of Namakkal, Tamil Nadu, India. The stem bark was dried in shade at room temperature and coarsely powdered using mechanical grinder. The coarsely powdered drug was then extracted successively with petroleum ether and ethanol for 24 hours. The extract was concentrated under reduced pressure. The dried extracts were stored under air tight containers.

Animals

Male Wistar albino rats (150-200g) were housed in a spacious cage for 10 days after getting approval from the “Institutional animal ethical committee” (Ethical committee IAEC reg no: 1158/ac/07/CPCSEA). During the experiment animals were fed standard chow diet. After randomized into various groups the rats were acclimized for 2 to 3 days in the new environment before initiation of the experiment. Animal had free access to food and drinking water till before 30 minutes of sampling.

Chemicals

Streptozotocin was purchased from Sigma Aldrich Co., Germany. Nicotinamide was purchased from Qualigens Fine Chemicals, Division of Glaxo, Mumbai, India.

Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test was performed in overnight fasted (8-h) normal animals. Rats were divided into four groups. Six fasted animals were used in each group. Rats divided into four groups (n=6) were administered 2% gum acacia solution, Ethanol extract of *Nyctanthes arbor-tristis linn* 250mg/kg and 500mg/kg and standard drug glibenclamide (0.25 mg/kg), respectively. Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro-orbital sinus at 0, 30, 60, 90 and 120 min of extract administration [6]. Fasting blood glucose levels were estimated by glucose oxidase-peroxidase reactive strips (Accu-check, Roche Diagnostics, USA).

Induction of experimental diabetes

The animal model of type-2 diabetes mellitus (NIDDM) was induced (23) in overnight fasted animals by a single intraperitoneal injection of 60 mg/kg STZ, 15 min after the i.p. administration of 120 mg/kg nicotinamide. Hyperglycemia was confirmed by the elevated blood glucose levels determined at 72 h and then on day 7 of the injection. Only rats confirmed with permanent NIDDM were used in the antidiabetic study [7].

EXPERIMENTAL DESIGN

All the animals were randomly divided into 5 groups. Group I was normal and used as control. Group II were used as diabetic control. Group III, IV and V were served as standard and extract treated groups 250mg/kg and 500mg/kg respectively. The blood was collected from tail vein for 0, 7, 14 and 21 days.

Estimation of biochemical parameters

Serum cholesterol, serum triglyceride, HDL was estimated by commercially available kids (Span diagnostics Pvt. Ltd. Surat, India).

Statistical Analysis

All the results are expressed as the mean± S.E.M. the results were analyzed for statistical significance using one way analysis of variance (ANOVA), comparison was done by using Dunnett's test. P values<0.5 were considered as significant and P<0.01 were considered as very significant.

RESULTS

Effect on Glucose Tolerance Test

The effects of ethanol extract of *Nyctanthes arbor-tristis linn* (250mg/kg & 500mg/kg) on glucose tolerance are shown in Table 1. The supplementation of *Nyctanthes arbor-tristis linn* improved the glucose tolerance in the fasted normal rats. When the rats were first injected with glucose, the rate of increase in the blood glucose level was the

same for normal and extract groups during the first 30 minutes but its rise was less for the ethanol extract group. After that serum glucose level lowered significantly (P<0.05) at 90 minutes and very significantly (P<0.01) lowered at 120 minutes in the ethanol group as compared to the normal control group. There was significant reduction (P<0.01) in the standard drug treated group compared to the control group.

Effect on Streptozotocin-Nicotinamide induced Diabetic Rats

Administration of streptozotocin (60mg/kg) and nicotinamide (120mg/kg) led to elevation of blood glucose. The anti-hyperglycaemic effects of the ethanol extract of bark of *Nyctanthes arbor-tristis linn* (250mg/kg, 500mg/kg) and glibenclamide (5mg/kg) on the blood sugar levels of diabetic rats are shown in Table 2. After daily treatment with 250mg/kg, 500mg/kg of ethanol extract of *Nyctanthes arbor-tristis linn* and glibenclamide 5 mg/kg led to a dose- dependent fall in blood sugar levels. The percent reduction of hyperglycemia was more significant (P<0.01) on 7^{th}, 14^{th}, and 21^{st} days after treatment with the 500mg/kg of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn*, as compared with the diabetic control group. The percent reduction of hyperglycemia was significant (P<0.05) on 7^{th}, 14^{th}, and 21^{st} day after treatment with the 250 mg/kg of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn*, as compared with the diabetic control group. The antihyperglycemic effect exhibited by 500 mg/kg of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* was slightly lower than the glibenclamide 5 mg/kg.

Effect on Serum Cholesterol, Triglycerides, HDL and Liver Glycogen Content The levels of serum cholesterol and triglycerides were increased very significantly and the levels of HDL were decreased in diabetic rats as compared with normal control rats. Treatment with ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* at the doses of 250mg/kg and 500mg/kg reduced the cholesterol and triglycerides level very significantly (P<0.01), when compared with the diabetic control group. The reduction in cholesterol and triglycerides levels in the extract-treated group was slightly higher at the dose of 500mg/kg as compared to standard drug glibenclamide. The level of HDL cholesterol was significantly (P<0.05) increased in the extract-treated group when compared to the diabetic control group. The HDL cholesterol improvement at the doses of 500 mg/kg of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* treatment was also slightly lower than the glibenclamide 5 mg/kg; the data are shown in Table 3.

DISCUSSION

Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This has led to an increase in the demand for natural products with antihyperglycemic activity and fewer side effects. The ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* exhibited dose-dependent antidiabetic property. The antidiabetic effect of ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* at the dose of 500 mg/kg is even slightly lower than glibenclamide 5mg/kg. Our results are supporting its use as folklore medicine for the treatment of diabetes. Plants may act on blood glucose through different mechanisms, some of them may have insulin-like substances and some may inhibit insulinase activity [8,9]. Stimulation of β-cells to produce

more insulin [9] and others may increase β-cells in the pancreas by activating regeneration of pancreatic cells [10]. Lipids play an important role in the pathogenesis of diabetes mellitus. Hyperlipidemia is a recognized consequence of diabetes mellitus demonstrated by the elevated levels of tissue cholesterol, phospholipids and free fatty acids [9-11]. Diabetes-induced hyperlipidemia is attributable to excess mobilization of fat from the adipose tissue due to the under utilization of glucose. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots since insulin inhibits the hormone sensitive lipase. On the other hand, glucagon, catecholamine, and other hormones enhance lipolysis. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease. The levels of serum cholesterol and triglycerides were raised in diabetic rats but which were lowered significantly with the treatment of stem bark of *Nyctanthes arbor-tristis linn*. It indicates that the ethanol extract of stem bark of *Nyctanthes arbor-tristis linn* is more useful in the treatment of diabetes as it has hypolipidemic effect. Moreover, its hypolipidemic effect could represent a protective mechanism against the development of atherosclerosis, which is usually associated with diabetes. The levels of HDL cholesterol were significantly increased in the extract treated group. Glycogen is the primary intracellular storable form of glucose and its levels in various tissues especially hepatic and skeletal muscle are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. Since destruction of β-cells of islets of Langerhans resulting in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for influx of glucose [12]. Moreover, alteration in muscle and hepatic glycogen content is normalized by insulin treatment. A normal level of glycogen reflects the normalization insulin levels.

Table: 1 Effect of EENA on Oral Glucose tolerance Test in Rats

Groups	Fasting	30 min	60 min	90 min	120 min
Control	**82.0 ± 3.16**	**115.75 ± 2.13**	**112 ± 3.84**	**109.0 ± 2.44**	**106.25 ± 2.17**
Diabetic + Glibenclamide (5mg/kg)	**77.0 ± 1.77**	**96.5 ± 2.10** [a]	**94.0 ± 1.95** [a]	**90.75 ± 1.65** [a]	**89.27 ± 1.03** [a]
Diabetic + EENA (250 mg/kg)	**8.25 ± 1.65**	**107 ± 0.85** [a]	**105 ± 1.19** [a]	**100.1 ± 1.95** [a]	**98.25 ± 1.65** [a]
Diabetic + EENA (500 mg/kg)	**76.25 ± 0.825**	**102.25 ± 1.25** [a]	**98.23 ± 1.25** [a]	**96.75 ± 2.32** [a]	**94.75 ± 1.795** [a]

Values are given as mean ± S.E.M for groups of six animals each. Values are statistically significant at a - **P<0.05, b - **P<0.01, C - ***P<0.001.*

Glucose (control) groups were compared with Extract treated and standard drug group.

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Table: 2: Effect of EENA on Blood glucose of control and experimental rats.

Groups	Treatment	Blood glucose level (mg/dl)			
		0 Day	7th Day	14th day	21st day
I	Control	92.16 ± 3.25	96.13 ± 2.73	94.8 ± 4.65	97 ± 1.81
II	Diabetic Control	365.83 ±1 .10^{C}	374.66 ± 4.38^{C}	390.2 ± 4.25^{C}	405.1 ± 7.95^{C}
III	Diabetic + Glibenclamide 5mg/kg	305 ± 4.59^{C}	250.4 ± 4.26^{C}	204.4 ± 5.24^{C}	139 ± 9.33^{C}
IV	Diabetic + EENA 250mg/kg	306.6 ± 4.02	265.4 ± 12.26^{C}	217.6 ± 9.67^{C}	178.5 ± 6.41^{C}
V	Diabetic + EENA 500mg/kg	307.83 ± 3.58	253.4 ± 7.54^{C}	202.4 ± 4.86^{C}	141.8 ± 3.42^{C}

Values are given as mean ± S.E.M for groups of five animals each. Values are statistically significant at (a - *P<0.05, b - P<0.01, C -P<0.001)*
Normal control groups were compared with diabetic control and EEWC – treated diabetic rats were compared with diabetic rats; Glibenclamide – treated diabetic rats were compared with diabetic control rats.

Table 3: Effect of EENA on Total cholesterol, Triglycerides, HDL control and experimental groups of rats

Groups	Total Cholesterol	Triglycerides	HDL Cholesterol
Control	132±3.1	82±1.68	48.61±1.86
Diabetic control	255.3±2.73^{c}	206.6±4.78^{c}	28.28±1.854^{b}
Diabetic + Standard Drug (5 mg/kg)	160.78±1.27^{b}	87.6±2.274^{b}	52.8±2.358^{b}
Diabetic + Ethanol Extract (250 g/kg)	180±2.31^{a}	119.4±2.239^{a}	38.17±1.77^{a}
Diabetic + Ethanol Extract (500 g/kg)	165.66±2.13^{b}	95.8±1.97^{b}	43.45±2.27^{b}

Values are given as mean ± S.E.M for groups of 5 animals each. Values are statistically significant at (*a - P<0.05, b - P<0.01, c- P<0.001)*
Normal control groups were compared with diabetic control and EEWC – treated diabetic rats were compared with diabetic rats; Glibenclamide – treated diabetic rats were compared with diabetic control rats

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