



HYPOGLYCEMIC ACTIVITY OF METHANOLIC EXTRACTS OF *NYCTANTHES ARBOR-TRISTIS* LINN. ROOT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

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. This research aims to investigate the hypoglycemic activity of methanolic extract of *Nyctanthes arbor-tristis* Linn. root in alloxan induced diabetic albino rats. A comparison was made between the action of *Nyctanthes arbor-tristis* Linn. methanolic extract and a known antidiabetic drug glibenclamide (0.5mg/kg p.o). The methanolic extract of *Nyctanthes arbor-tristis* Linn. root was administered orally at different doses to normal rats. The methanolic extract at 500 mg/kg dose level exhibited significant ($p < 0.05$) hypoglycemic activity. The antidiabetic activity of methanol extract of root of *Nyctanthes arbor-tristis* linn is comparable to that of diabetic control animals. It is concluded that methanol extract of root of *Nyctanthes arbor-tristis* linn possess safe and strong antidiabetic activity.

Keywords: *Nyctanthes arbor-tristis* Linn, Alloxan, Glibenclamide, Anti-diabetic activity.

INTRODUCTION

Herbs have recently attracted attention as health beneficial foods and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases including liver disease¹, ischemia, perfusion injury, atherosclerosis, acute hypertension, hemorrhagic shock, diabetes mellitus and cancer with relatively little knowledge regarding their modes of action². The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time.

The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential³. *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 Chhattisgarh 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^{4,6}. Leaves are also used in the enlargement of spleen. The objective of the present work is aimed to evaluate the effect of ethanolic extract of *N.arbortristis* roots on alloxan induced diabetic rats.

MATERIALS AND METHODS

Animals

Albino wistar male rats weighing 150-200g was used for the present study. They were maintained in the animal house of School of pharmacy, SGVU, Jaipur for experimental purpose. The animals were maintained under controlled conditions of temperature ($23 \pm 2^\circ\text{C}$), humidity ($50 \pm 5\%$) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of School of pharmacy, SGVU, Jaipur. According to prescribed guidelines of CPCSEA, Government of India.

Plant Material

Roots of the plant *N. arbor-tristis* Linn were obtained and identified from authentic sources. The roots of the plant

Nyctanthes arbor-tristis Linn were collected from Haridwar and authenticated by Rajasthan University, Jaipur. The collected roots were dried in shade, crushed to coarse powder and used for further studies.

Preparation of extract

50g root powders were extracted with 400ml of methanol for 18h by hot continuous extraction method. The methanolic extract was filtered and partitioned by using petroleum ether to remove impurities. The solvent was evaporated under reduced pressure and dried in vacuum. The dried extract of *Nyctanthes arbor-tristis* Linn thus obtained was used for the assessment of hypoglycemic activity. The extracts were subjected to preliminary qualitative tests^{7,8} to identify the various phytoconstituents present in roots.

Ld₅₀

The extract of *N. arbor-tristis* Linn root was found to be safe for further biological studies as no toxic effect and lethality was observed up to 3000 mg/kg per oral in rat. Only the consumption of food was increased by 20% in the dose of 2000 and 3000 mg/kg during 4h but remaining normal afterwards.

Hypoglycemic activity in normal rats

Twenty-four albino rats weighing 150-200g were fasted for 18h and were divided into four groups of six animals in each. The groups included **i) (vehicle control)** received 5% gum acacia in normal saline, 1ml/200g rat. **ii) (Test drug I)** received 250mg/kg, p.o. 5% w/v *N. arbor* root extract, and 1ml/200 g rat. **iii) (Test drug II)** received 500 m/kg, p.o. 10% w/v *N. arbor* root extract, and 1ml/200 g rat. **iii) (Standard)** received Glibenclamide (0.5 mg/kg p.o. 10% w/v, 1ml/200 g rat). One milliliter of blood from the tail of each rat was collected at '0' hour. At two hours of treatment, blood samples were collected again from the treated animals and blood glucose was estimated by glucose estimated method⁹.

Hypoglycemic activity in diabetic rats

Albino rats (n=44) were fasted for 48h. Diabetes was inducing by administering^{10,13,14} freshly prepared alloxan monohydrate 2.4% in normal saline subcutaneously at a dose of 120 mg/kg, body weight as single dose¹¹. After 72h of alloxan, 18 h fasting blood was collected from those that survived (n=34)¹² sugar estimated by glucose oxidase method. Twenty four diabetic rats with blood glucose level of 300-500 mg% were selected and were divided into four groups of six each.

Table 1: Hypoglycemic activity of *N. arbor* root extract in normal rats

Groups	Fasting	2 h after Treatment
Control (2%gum acacia)	78.05 ± 0.03	71.73 ± 0.05
Test I <i>N. arbor</i> (250mg/kg)	73.55 ± 0.07	60.86 ± 0.05*
Test II <i>N. arbor</i> (500mg/kg)	70.76 ± 0.08	52.63 ± 0.03**
Std Glibenclamide (0.25 mg/Kg)	69.76 ± 0.25	42.45 ± 0.04**

Values are mg (%), mean ± SD, n=6 in each group, *p<0.01, **p<0.001 as compare to respective control.

Table 2: Hypoglycemic activity of *N. arbor* root extract in diabetic rats

Groups	Fasting	2 h after Treatment
Control (2%gum acacia)	301.7 ± 35.53	217.16 ± 23.05
Test I <i>N. arbor</i> (250mg/kg)	290.54 ± 23.45	120.66 ± 9.80**
Test II <i>N. arbor</i> (500mg/kg)	289.23 ± 27.25	96.83 ± 5.23**
Std Glibenclamide (0.25 mg/Kg)	312.33 ± 32.25	80.66 ± 4.22**

Values are mg (%), mean ± SD, n=6 in each group, *p<0.01, **p<0.001 as compare to respective control.

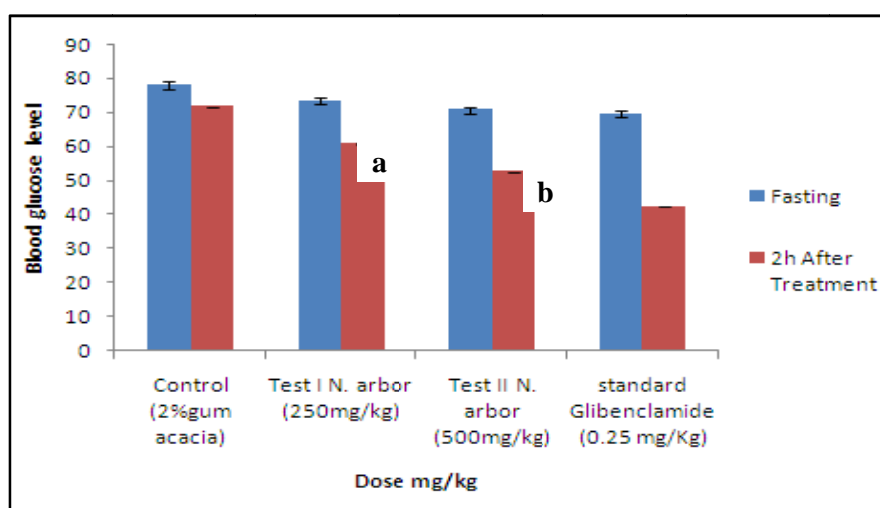


Fig. 1: Blood glucose level in normal rats

a=p<0.001 Vs Control, b=p<0.001 Vs Control

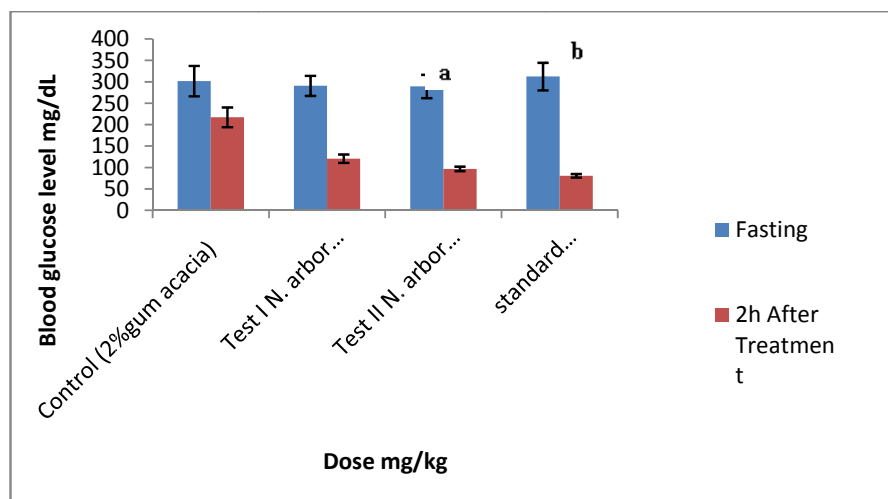


Fig. 2: Blood glucose level in diabetic rats

a=p<0.001 Vs Control, b=p<0.001 Vs Control

RESULTS

The plant extract of *N. arbor-tristis* Linn roots methanolic extract showed hypoglycemic activity by reducing blood glucose level significantly. It is also much effective when compare with the standard drug Glibenclamide. It reduces blood glucose level after seven days at the 500 mg/kg in rats compare with standard drug. We found that methanolic extract of plant *N. arbor-tristis* Linn roots is more effective in reducing the blood glucose level compare to the standard drug (Glibenclamide).

The hypoglycemic activity of methanolic extract of *N. arbor-tristis* Linn root in normal (non diabetic) and diabetic rats is shown in Table. The test drug, at a dose of 500 mg/kg, p.o. significantly lowered the blood, at 2h. However, the activity of the standard drug, glibenclamide (0.5mg/kg/day), was more pronounced ($P < 0.001$). In alloxan induced diabetic albino rats, *N. arbor-tristis* Linn at a dose of 250 and 500 mg/kg/day and standard drug glibenclamide (0.5mg/kg/day) for seven days was highly significant ($P < 0.001$) in comparison with control group (Figure 1 and 2). However, in diabetic rats the hypoglycemic effect of the test drug at 250 mg/kg was significantly less than the standard drug glibenclamide.

Statistical Analysis

Results are expressed as mean \pm SD. The differences between experimental groups were compared by one-way Analysis of Variance (ANOVA) followed by Bonferroni's test. The results were considered statistically significant when $P < 0.05$.

DISCUSSION

The methanol extract of root of *Nyctanthes arbor-tristis* linn exhibited dose-dependent antidiabetic property. The antidiabetic effect of ethanol extract of root 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^{15,16}. Stimulation of β -cells to produce more insulin¹⁶ and others may increase β -cells in the pancreas by activating regeneration of pancreatic cells¹⁷.

The mechanism of alloxan diabetes has been the subject of many investigations and it is now generally accepted that free radicals are selectively involved in the initiation of the damage that ultimately leads to β cells death¹⁵. Therefore, the pancreas is especially susceptible to the action of alloxan induced free radical damage. Many substances have been shown to ameliorate the diabetogenicity of alloxan in animals, which protect by reacting with free radicals formed from alloxan during its interaction with β cells, or prevent radical formation¹⁸. Recently, it was reported that the *Nyctanthes arbor-tristis* linn extract, exhibited significantly radical scavenging activity and thus antioxidant activity and the present finding indicates that administration of *Nyctanthes arbor-tristis* linn root confirms the possibility that the major function of the extract is on the protection of vital tissues including the pancreas, thereby reducing the causation of diabetes in these animals¹⁹. Therefore, protective effect of *Nyctanthes arbor-tristis* linn extract on pancreas of alloxan induced diabetic rats could be attributed directly to scavenging activity and for more extent to the regenerative properties of the extract.

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