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HYPOGLYCEMIC EFFECT OF *NYCTANTHES ARBORTRISTIS* LINN EXTRACTS IN NORMAL AND STREPTOZOTOCIN-INDUCED DIABETIC RATS

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Nyctanthes arbortristis Linn is widely used in Bangladesh and South Asia. The plant has a long history of traditional medicine use in the treatment of various ailments. This study examined the hypoglycemic effects of N. arbortristis Linn ripe seed and leaves extracts in rat models. Aqueous and 2% ethanol extracts of seed and leaves of N. arbortristis were used at a dose of 1.25 g/kg body weight. Male Long-Evans rats, bred at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) animal house, weighing between 180–200 g were used in the study. Type 1 and Type 2 diabetic models of rats were induced with intraperitoneal injection of streptozotocin using conventional methods. Experiments were done in normal, Type 1 and Type 2 model rats with a single feeding in different prandial states. Control rats received water. Glibenclamide and insulin were given to positive control groups. Serum glucose was measured by glucose-oxidase methods. Orally administered ripe seed extracts had significant effect on blood glucose level in fasting condition ($p<0.005$) as well as when the extract fed simultaneously with glucose ($p<0.001$) and 30 min before glucose load ($p<0.05$) in diabetic and normal model rats compared with control group. The leaves extract also reduced blood glucose level significantly in Type 1 model rats ($p<0.05$) compared to control rats. The extracts of the plant of N. arbortristis exhibited hypoglycemic effects which merits further exploration both chemically and biologically.

Keywords: *Nyctanthes arbortristis* Linn, Leaf, Ripe seed, Hypoglycemic

INTRODUCTION

Diabetes is a life-long disease effecting mankind all over the world. Traditional preparations from plant sources are widely used almost everywhere in the world to treat this disease. Therefore, alternative strategies to the current modern pharmacotherapy of diabetes mellitus are urgently needed (WHO 2002), because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the

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enormous cost and poor availability of modern therapies to many rural populations in developing countries. To the best of our knowledge there has been no published report on the antidiabetic properties of *Nyctanthes arbortristis* Linn.

N. arbortristis Linn (Seuli) (Fig. 1) is a shrub cultivated as a garden plant throughout Bangladesh and in the sub-himalayan region. It is a C_3 plant (Rao and Kodandaramaiah 1982). The leaves are used extensively in Ayurvedic and Unani medicine for the treatment of chronic fever, rheumatism, intestinal worms and as a laxative, cholagogue, diuretic, diaphoretic, expectorant and for antimoebic purposes (Singh *et al*. 1995; Srivastava *et al*. 1990; Chopra *et al*. 1956). The seeds of this plant are known for their use in Ayurvedic system of medicine for throat, leprosy, eye diseases, skin infections and intestinal worm infection treatment (Singh and Jindal 1985). In accordance with WHO (1980) recommendation, we therefore undertook this work to study the hypoglycemic effects of *N. arbortristis* ripe seeds and leaves extracts in normal, Type 1 and Type 2 diabetic model rats.

METHODS

Plant Materials

Ripe seeds and fresh leaves of *N. arbortristis* were collected from Dhaka city, Bangladesh from January to February, 2003. The plant was identified by a taxonomist, Dr. Md. Salar Khan of Bangladesh National Herbarium Centre, Dhaka, Bangladesh and a voucher specimen (DACB accession number-35121) was kept in the Bangladesh National Herbarium Centre, Dhaka, Bangladesh.

Preparation of the Extracts

Ripe seeds and fresh leaves of *N. arbortristis* were cleaned and air-dried followed by drying in an oven at 40°C. The dried ripe seeds and leaves were ground to coarse powder and stored separately. The powdered ripe seeds (1.9 kg) extracted with distilled water for 8 days at room temperature and the powdered leaves (0.75 kg) were extracted with 2% ethanol for 5 h at 70°C by the Soxhlet method. After extraction, filtrates were evaporated at 50°C in a vacuum dryer to give final yields of 96.6 g and 46.5 g, respectively. The extracts were stored in the refrigerator at –2°C until use.

Experimental Animals

Adult male Long Evans rats, weighing (180–200 g), were used throughout the study. The animals were bred at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) animal house. All animal experiments were approved by the Ethical Review Committee of Diabetic Association of Bangladesh (Ref no.18/03/A/ERCDAB) and followed the guideline of the International Guidelines for Handling of Laboratory Animals (Derrell 1996). The rats were housed 5 rats per cage at a temperature of 22±2°C with 12 h light/12 h dark cycle with access to feeding with standard pellet diet and water *ad libitum*. The rats were fasted 12 h before and during the whole period of blood sampling.

Fig. 1: *Nyctanthes arbortristis* Linn (Seuli).

Induction of Type 1 Diabetes

Type 1 diabetes was induced by a single intra-peritoneal injection of streptozotocin (Upjohn Company, Kalamazoo, Michigan, USA) dissolved in 0.1 M sterile citrate buffer, pH 4.5, at a dose of 60 mg/kg. Fasting blood glucose was determined on the 7th day after induction, and rats with fasting blood glucose of 18 mmol/L or more were considered for the experiment.

Induction of Type 2 Diabetes

Type 2 diabetes was induced by a single intra-peritoneal injection of streptozotocin (90 mg/kg) to 48 hour-old pups as described by Bonner-Weir *et al.* (1981). Three months later oral glucose tolerance test was performed and rats with serum glucose of 8 mmol/L or more were considered for the experiments.

Control Group

Rats in negative control groups received water (10 mL/kg) daily whereas the positive control group in Type 1 model received insulin (5 µL/kg) once daily and Type 2 model received glibenclamide (5 mg/kg) daily. Normal rats also received glibenclamide (5 mg/kg) daily.

Effect of the Ripe Seed and Leaf Extracts on the Fasting Glucose Levels of Rats

Rats were fasted for 24 h before the experiment. The ripe seed and leaf extracts (1.25 g/kg) were administered orally to rats. The blood samples were withdrawn for measurement of the glucose level after cutting the tail tip of rats under mild ether anesthesia at 0 min, 60 min and 120 min.

Effect on Blood Glucose Levels of Rats when the Ripe Seed and Leaf Extracts were Fed Simultaneously with Glucose

The rats were kept fasting overnight (at least 12 h), without free access of water, before testing the blood glucose level. Rats were kept in an airtight jar or desiccator with saturated ether vapour for 1 min for anesthesia, before fed with the extracts. The extracts (1.25 g/kg) were administered orally to rats, simultaneous with glucose (2.5 g/kg) and blood samples were drawn at 0 min, 30 min and 75 min.

Effect on Blood Glucose Levels of Rats when the Extracts were Fed 30 Min before Glucose Load

Extracts (1.25 g/kg) were fed by metallic smooth tubes to the rats fasted 12 h under mild ether anesthesia. All rats were given glucose (2.5 g/kg) 30 min after the administration of extracts. Blood samples were drawn at 0 min, 60 min and 105 min.

Blood Collection and Determination of Serum Glucose Level

Blood was collected by amputation of the tail tip under mild ether anesthesia. The serum was separated by centrifugation and glucose levels in the serum samples were estimated on the same day by an automated colorimetric method (Peridochrom Glucose GOD-PAP, Boehringer, Germany) at absorbance of 510 nM (Nahar *et al.* 2000).

Data and Statistical Analysis

All analyses were done using the Statistical Package for Social Sciences (SPSS). Results were expressed as mean±standard error (mean±SE). Statistical comparisons with the

control were made by one way ANOVA test (Duncan's new multiple-range test post hoc). The values with $p<0.05$ were considered significant.

RESULTS

In Table 1, fasting blood glucose was 20.3±1.0 to 22.7±2.5 mmol/L on the 7th day after induction. In Type 2 diabetic model rats fasting glucose level was only marginally higher (6.9±0.2 to 8.5±0.5 mmol/L at 0 min) indicating the presence of functioning β-cells. The aqueous extract of *N. arbortristis* ripe seeds significantly lowered fasting blood glucose levels (7.0±0.1 mmol/L) in Type 2 diabetic rats ($p<0.05$ at 120 min). Glibenclamide had significant effect in reducing blood glucose in normal and Type 2 diabetic model rats at 120 min ($p<0.05$) and 60 min ($p<0.05$), 120 min ($p<0.01$) in this condition, respectively.

The leaves extract did not decrease the blood glucose significantly in normal rats at 60 min (5.9±0.5 mmol/L) and 120 min (5.2±0.6 mmol/L) compared to normal control rats. Leaf extract did not show any hypoglycemic activity in Type 1 and Type 2 rats in fasting condition. On the other hand, the seed extract did not significantly lower fasting blood glucose levels in normal and Type 1 diabetic model rats in fasting condition.

Table 1: Effect of *N. arbortristis* Linn aqueous extract of ripe seeds and 2% ethanol extract of leaves on fasting serum glucose levels of normal and diabetic models (Type 1 and Type 2) rats.

Group	0 min (mmol/L)	60 min (mmol/L)	120 min (mmol/L)
Normal rats			
Water control (10 mL/kg) (n=7)	6.1±0.3	5.7±0.4	5.7±0.3
Glibenclamide (5 mg/kg) (n=7)	6.6±0.3	4.6±0.6	4.2±0.4*
N. arbortristis ripe seed (n=6)	6.1±0.3	6.3±0.4	5.3±0.6
N. arbortristis leaves (n=7)	6.3±0.4	5.9±0.5	5.2±0.6
Type 1 diabetic model rats			
Water control (n=6)	22.1±2.9	22.0±2.5	20.3±1.3
Insulin control (n=6)	20.3±1.0	3.6±0.6***	2.4±0.1***
N. arbortristis ripe seed (n=7)	22.7±2.5	22.4±2.7	21.1±2.5
N. arbortristis leaves (n=7)	21.9±2.2	20.9±2.3	22.3±3.0

(*continued on next page*)

Table 1: (*continued*)

Group	0 min (mmol/L)	60 min (mmol/L)	120 min (mmol/L)
Type 2 diabetic model rats			
Water control (n=6)	8.5±0.5	8.4±0.3	7.9±0.2
Glibenclamide control (n=6)	8.4±0.3	7.3±0.3*	6.8±0.2 **
N. arbortristis ripe seed (n=7)	7.2±0.2	8.8±0.1	7.0±0.1*
N. arbortristis leaves (n=7)	6.9±0.2	7.8±0.1	7.5±0.1

Notes: Values are expressed as mean±SE; n = number of rats; *p<0.05, **p<0.01, ***p<0.001 compared to control rats. Statistical significant test for comparisons were done by one way ANOVA followed by Duncan's new multiple-range test.

Postprandial Condition with Simultaneous Glucose Load

In this experimental situation (Table 2), aqueous extract of *N. arbortristis* leaves significantly opposed the rise of blood glucose (25.9±2.8 mmol/L) of Type 1 diabetic rats at 75 min (p<0.05) compared to control diabetic rats. In Type 2 diabetic rats, ripe seed extract showed significant antihyperglycemic effect (9.0±0.1 mmol/L) at 75 min (p<0.01) compared to control diabetic rats. Glibenclamide had significant hypoglycemic effect (5.2±0.6 mmol/L) in normal rats (p<0.01) at 75 min, but did not show significant effect of lowering blood glucose in Type 2 diabetic model rats in this condition. On the other hand, in normal and Type 2 diabetic model rats, glibenclamide did not show significant effect opposing the rise of blood glucose at the time point, i.e. 30 min.

In normal rats, the oral administration of postprandial condition with simultaneous glucose load and the extracts did not significantly affect the lowering of blood glucose levels at 30 min and 75 min (i.e. leaves extract 7.6±0.4 mmol/L and 6.9±0.4 mmol/L; ripe seed extract 7.6±0.5 mmol/L and 7.1±0.4 mmol/L). In Type 1 diabetic model rats, *N. arbortristis* ripe seed extracts did not show significant effect opposing the rise of blood glucose at 30 min and 75 min. Similarly, in Type 2 diabetic model rats, *N. arbortristis* leaves extracts did not show significant effect of lowering blood glucose levels at 30 min (15.4±1.4 mmol/L) and 75 min (16.2±1.0 mmol/L).

Postprandial Condition when the Extract was Fed to Rats 30 Min before Glucose Load

Table 3 shows the effect of postprandial condition when the extract was fed 30 min before glucose load administration. The ripe seeds extract of *N. arbortristis* significantly opposed the rise of blood glucose (6.7±0.3 mmol/L) at 60 min in nondiabetic rats (p<0.05) compared to control rats. On the other hand, the leaves extract did not show significant lowering of blood glucose in normal rats under this condition. Glibenclamide had significant antihyperglycemic effect (5.7±0.2 and 4.1±0.2 mmol/L) in normal rats at 60 min (p<0.01) and 105 min (p<0.001) in this condition, respectively.

Under this condition, *N. arbortristis* ripe seeds and leaves extracts had no significant antihyperglycemic effect in Type 1 and Type 2 diabetic model rats. There was no significant change in lowering blood glucose in Type 2 glibenclamide control diabetic model rat in this condition.

Table 2: Effect of *N. arbortristis* Linn aqueous extract of ripe seeds and 2% ethanol extract of leaves on serum glucose levels of normal and diabetic models (Type 1 and Type 2) rats when the extract was fed simultaneously with glucose.

Group	0 min (mmol/L)	30 min (mmol/L)	75 min (mmol/L)
Normal rats			
Water control (n=6)	5.9±0.5	7.4±0.5	7.6±0.4
Glibenclamide control (n=7)	6.6±0.4	6.8±0.5	5.2±0.5**
N. arbortristis ripe seed (n=7)	6.9±0.3	7.6±0.5	7.1±0.4
N. arbortristis leaves (n=7)	6.6±0.4	7.6±0.4	6.9±0.4
Type 1 diabetic model rats			
Water control (n=6)	22.3±1.9	34.2±1.3	32.0±0.8
Insulin control (n=6)	22.5±0.5	22.1±0.7***	11.4±1.2***
N. arbortristis ripe seed (n=10)	24.5±1.2	32.5±1.2	27.1±2.3
N. arbortristis leaves (n=8)	22.3±0.8	30.6±1.1	25.9±2.8*
Type 2 diabetic model rats			
Water control (n=6)	8.1±1.0	17.1±0.5	15.9±0.5
Glibenclamide control (n=6)	8.3±0.8	19.2±1.4	17.0±1.2
N. arbortristis ripe seed (n=8)	8.3±0.9	15.4±1.1	9.0±0.1**
N. arbortristis leaves (n=7)	7.1±0.9	15.4±1.4	16.2±1.0

Notes: Values are expressed as mean±SE; n = number of rats; *p<0.05, **p<0.01, ***p<0.001 compared to control rats. Statistical significant test for comparisons were done by one way ANOVA followed by Duncan's new multiple-range test.

Table 3: Effect of aqueous extract of *N. arbortristis* Linn ripe seed and 2% ethanol extract of leaves on serum glucose levels of normal and diabetic models rats (Type 1 and Type 2) when the extract was fed 30 min before glucose load.

Group	0 min (mmol/L)	60 min (mmol/L)	105 min (mmol/L)
Normal rats			
Water control (n=6)	6.1±0.2	7.9±0.2	7.3±0.3
Glibenclamide control (n=7)	6.1±0.3	5.7±0.2**	4.1±0.2***
N. arbortristis ripe seed (n=8)	6.3±0.4	6.7±0.3*	7.3±0.3
N. arbortristis leaves (n=6)	5.9±0.4	7.4±0.3	6.8±0.2
Type 1 diabetic model rats			
Water control (n=5)	22.2±4.6	30.4±3.3	26.7±2.8
Insulin control (n=6)	21.4±3.2	9.5±4.1***	5.2±2.0 ***
N. arbortristis ripe seed (n=7)	19.6±3.3	27.7±3.0	24.4±2.8
N. arbortristis leaves (n=6)	19.5±6.1	27.3±3.8	27.2±4.0
Type 2 diabetic model rats			
Water control (n=6)	9.2±0.5	18.6±1.6	17.6±1.0
Glibenclamide control (n=7)	9.0±0.5	16.8±1.9	15.0±1.7
N. arbortristis ripe seed (n=5)	9.4±0.3	19.5±1.8	19.0±1.7
N. arbortristis leaves (n=6)	9.8±0.8	17.6±1.7	19.4±1.6

Notes: Values are expressed as mean±SE; n = number of rats; *p<0.05, **p<0.01, ***p<0.001 compared to control rats. Statistical significant test for comparisons were done by one way ANOVA followed by Duncan's new multiple-range test.

DISCUSSION

Streptozotocin-induced hyperglycemic has been described as a useful experimental model to study the activity of hypoglycemic agents (Szkudelski 2001). Streptozotocin selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state (Szkudelski 2001; Kamtchouing *et al.* 1998). At a postprandial state when the

extract (1.25 g/kg) was fed simultaneously with glucose (2.5 g/kg), the leaves extract produced significant reduction ($p<0.05$) in the blood glucose concentration of Type 1 diabetic rats at 75 min. This effect indicates that the extract has hypoglycemic activities, either acting through stimulation of insulin release from pancreatic β-cells and/or acting either at the gut level or at the peripheral tissues. Analysis of the nature of the action of the plant leaves extract (no effect in fasting state, but significant effect when fed simultaneously with the glucose load) indicates its probable effect at the glycogen synthesis level, since glycogenesis is the predominant mechanism at fed state in contrast to gluconeogenesis which is characteristically activated in diabetic animals (Felig and Bergman 1990).

Glibenclamide treatment (5 mg/kg) was not as effective in reducing blood glucose in STZ-diabetic rats as in normoglycaemic rats. It has been reported that glibenclamide was not effective when destruction of β-cells has occurred and hence more effective in moderate diabetic rats than in severe diabetic animals (Hosseinzadeh *et al*. 2002; Cetto *et al*. 2000; Sharma *et al*. 1997). The acute hypoglycemic effect of glibenclamide in rats has been shown from the stimulation of insulin release from the residual β-cells and inhibition of glucagon secretion (Moller 2001). But, in Type 2 glibenclamide control model rats on fasting state, significant lowering of blood glucose occurred at 60 min ($p<0.05$) and 120 min ($p<0.001$). This result (Type 2 glibenclamide control model rats on fasting state) opposed the observation in fasting condition. Glibenclamide might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis in fasting condition.

N. arbortristis ripe seed extract had significantly opposed the rise of blood glucose on the fasting state in Type 2 diabetic model rats indicating that the extract possesses hypoglycemic activities, which acts by releasing of insulin from pancreatic β-cells.

In this study, it was observed that administration of *N. arbortristis* ripe seed in postprandial states (Type 2 and normal rats) to both types of rats lower their blood glucose which was also reflected in their sugar level. Activity in this prandial state is probably due to a systemic action, i.e. as a result of the stimulation of β-cells and subsequent release of insulin or enhancement of insulin action.

CONCLUSION

Based on the results of this study, it may be concluded that *N. arbortristis* ripe seeds and leaves extracts show interesting possibilities as a source of oral hypoglycemic agents. We believe that this plant must be considered as an excellent candidate for future studies on determining the mechanisms of its hypoglycemic activity, as well as for the isolation and identification of active hypoglycemic substances.

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