Pharmacognosy Magazine
ISSN: 0973-1296
Vol 4, Issue 16, Oct-Dec, 2008

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article

Antidiabetic Activity of *Nyctanthes Arbortristis*

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ABSTRACT

The purpose of the present investigation was to assess the antidiabetic property of *Nyctanthes arbortristis* leaves and flowers chloroform extract. In the present study antidiabetic properties of *Nyctanthes arbortristis* we investigated by hypoglycemic effect, potentiation action of exogenous insulin, oral glucose tolerance test and streptozotocin-induced diabetic rat model. The *Nyctanthes arbortristis* exerted hypoglycemic effect at relatively high dose 8 gm/kg of leaves and flowers chloroform extracts treated rats, significantly ($P<0.05$, $P<0.01$) lowered blood serum glucose levels, compared to 0 hrs. The maximum reduction in serum glucose levels observed at 5 hrs in flowers extract. The lower doses (50, 100 and 200 mg/kg) tested for potentiation action of exogenous insulin, oral glucose tolerance, streptozotocin-induced diabetic rat model. Oral glucose tolerance test was carried out in fasted rats by administering 2 gm/kg of glucose after administration of extract, the administration of extract significantly improved ($P<0.05$, $P<0.01$ $P<0.001$) compared to control (glucose 2 gm/kg) glucose tolerance test, which is comparable to glibenclamide 10 mg/kg treated group except 50 mg/kg of leaves extract. The potentiation action of exogenous Insulin was evaluated by administration of Insulin (1 unit/kg, i.p) after the administration of extract. The administration of extract in all dose significantly ($P<0.05$, $P<0.01$ $P<0.001$) potentiated exogenous action of Insulin, when compared to 0 hrs of treatment. The animals were made diabetic by streptozotocin (55 mg/kg, i.p) after confirming the diabetes level more than 300 mg/dl the chloroform extract from leaves and flower of *Nyctanthes arbortristis* (50, 100, 200 mg/kg) were used for 27 days in diabetic rats. The extract significantly ($P<0.05$, $P<0.01$, $P<0.001$) lowered serum glucose levels in treated rats when compared with control (vehicle treated diabetics). The antidiabetic activities of the leaves and flowers chloroform extract were comparable to glibeclamide at 10 mg/kg orally (positive control). In contrast, the flower extract shown more significant at 27 day of treatment, without significantly influencing on other days may be due to handling errors. This study confirms the significant antidiabetic activity of *Nyctanthes arbortristis* in flowers than leaves.

KEY WORDS: - *Nyctanthes arbortristis*, hypoglycemic, glucose tolerance test, Insulin action potentiation, and streptozotocin, diabetes, antidiabetic activity

INTRODUTION

Diabetes is the world's largest endocrine disease with deranged carbohydrate, fats and protein metabolism. As per WHO report, approximately 150 million people have diabetes mellitus world wide, and this number may well double by the year 2025. Statistical projection suggests that the number of diabetics will rise from 15 million in the year 1995 to 57 million in 2025, making India apart the country with the highest number of diabetics in the world. Although many drugs and interventions are available to manage diabetics, these are expensive for a developing country like India apart from their inherent adverse effects. Therefore, it is necessary to look for new avenues to manage this major health problem (1). As part of the pathogenesis of non insulin-dependent diabetes mellitus (NIDDM), skeletal muscle, liver and adipose tissues become resistance to the hormonal effect of insulin, which in turn leads to decreased insulin-mediated glucose disposal, hepatic glucose overproduction and a marked increase in lipolysis (1). An addition to the above, hyperinsulinemia is a central pathophysiological feature of NIDDM and has been shown to play a key role in the disease evaluation and macrovascular complication (1). The plants kingdom has become a target for the search by multinational drugs and biological active lead compound. Ethnobotanical

information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (2).

Hence, the present study was under-taken to explore antidiabetic activity of *Nyctanthes arbortristis* of different extracts on normal and streptozotocin induced diabetic rats. *Nyctanthes arbortristis Linn.* (Family: *Oleaceae)* commonly known as Harsinger or Night jasmine, is widely used as a decoction of leaves by Ayurvedic physicians for the treatment of diabetes, arthritis, obstinate, sciatica, malaria, intestinal worms and as tonic, cholagogue and laxative (3-6). The leaves have also been found to exhibit activity against *Plasmodium falciparum, Leishmania donovani and Entamoeba* histolytia (7), anti-inflammatory and antioxidant activity (8, 9). The isolated arbortristoside-A from the ethanolic extract of its seeds, shown anti-inflammatory and analgesic activity (10). *Nyctanthes arbortristis* have shown pro and anti-inflammatory cytokines by water-soluble ethanol extracts (11). Two pure compounds isolated from the plant *Nyctanthes arbortristis* were tested against Encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV) (12). *Nyctanthes arbortristis* leaves extract prevented silica-induced early fibrogenic reactions like, congestion, edema and infiltration of nucleated cells in the interstitial alveolar spaces, and thickening of alveolar septa in mouse lung (13), Iridoid glucosides (arbortristosides-A [I], B [2], C [3], and 6beta-hydroxy-loganin [4] shown antileishmanial activity in both *In vitro* and *In vivo* test systems (14), The phytochemical analysis of leaves of *Nyctanthes arbortristis* reveals the presence of ß-amyrin, ß-sitosterol, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid, nyctanthic acid, friedlin lupeol and oleanolic acid and 6ß-hydroxylonganin irioid glucosides-arborsides A, B and C (12).

MATERIAL AND METHOD

Nyctanthes arbortristis leaves and flower were collected from widely growing plants in the region of north Karnataka in the months of Sept-October 2005. The plant material was dried in shade and coarsely powdered and extracted with petroleum ether to (40-60^0 C) defat followed by benzene, chloroform, ethyl acetate and methanol by solvent extraction for 24 hrs/cycle. The extract was concentrated under rotary evaporator and dried in lyophilizer (Mini Lyotrap, Serial No. J8199/5, LET Scientific LTD, UK). The extracts were formulated as suspension in distilled water using 5% Tween-80, as suspending agent (15). *Wistar albino* rats (150-200 gm) of either sex were obtained from the central animal house S.N. Medical College, Bagakot, Karnataka and acclimatized to laboratory condition for one week and were given uniform diet (Food-pellet). Study design was cleared by Institutional Animals Ethics Committee. When different extracts were tested for hypoglycemic activity on normal rats, the chloroform extract was found active (dose was selected based on previous study (4, 6), whereas other extracts were found to be inactive. Hence chloroform extract was selected for the study. The qualitative test indicated the presence of alkaloids, flavonoid in the leaves and flower of chloroform extract of plant.

Evaluation of Hypoglycemic Activity (16, 18, 20)

The acclimatized animals were fasted for 24 hrs with water *ad libitum,* fasted animals were divided into three groups of six rats. Group 1 served as control, received 0.5ml of 5% Tween 80. Group 2 and 3 received *Nyctanthes arbortristis* flower and leaves chloroform extract at dose of 8 gm/kg respectively, the dose administered after withdrawing the initial (0 hrs) of blood sample and at on interval 1½, 3, 5 hrs after the flower and leaves extract administration. Blood samples were colleted from retro-orbital plexus under anesthesia, and were centrifuged at 1000 g for 15 min to obtained serum, and used for estimation of glucose by using OGENT Glucose kit (Manufactured by Span diagnostic, LTD) using star-21plus semi-autoanalyser.

Potentiation action of exogenous insulin (16)

The acclimatized animals were fasted for 24 hrs with water *ad libitum,* fasted animals were divided into eight groups of six rats. Group 1 served as control, received 0.5 ml of 5% Tween 80. Group. No. 2 received Insulin (1 Unit/kg, i.p) and Group No. 3-8 received the leaves and flower chloroform extract (50, 100, 200 mg/kg) respectively to rats after withdrawing the initial (0 hrs) and after 30 min of extract administration, the groups were treated with Insulin (1 Unit/kg) and blood samples were collected on interval of 30 min, 1, 2 hrs after extract administration, Serum glucose was estimated by repeating the above procedure.

Glucose tolerance test (17-18)

The acclimatized animals were fasted for 24 hrs with water *ad libitum,* fasted animals were divided into seven groups of six rats. Groups No. 1 served as control received distilled water. Groups No. 2 received Glibenclamide at an oral dose 10 mg/kg and groups 3-7 received Chloroform extract leaves and flower and at the dose of (50, 100, 200 mg/kg) respectively, after withdrawing the initial (0 hrs) of blood samples and

after 30 min of extracts administration, the rats of all groups were orally treated with 2 g/kg glucose. Blood samples were collected at the interval of 30, 90, 180 min, after glucose loading, from retro-orbital plexus under anesthesia, and were centrifuged at 1000 g for 15 min to obtain serum was used for estimation of glucose using OGENT Glucose kit (Manufactured by Span diagnostic LTD) using star-21plus semi-autoanalyser.

Evaluation of anti-diabetic activity (2, 16, 19)

The acclimated animals were kept fasting for 24 hrs with water *ad libitum*, on first day blood serum glucose levels were estimated before administering streptozotocin. The streptozotocin (Sigma chemical Co., U.S.A) freshly was dissolved in citrate buffer (pH 4.5) and made diabetic by injection of a single dose 55 mg/kg intraperitonally. Streptozotocin-treated rats were given 5% of glucose in drinking water for the first 24 hrs encounter any initial hypoglycemia. On the third day the animals were checked for serum blood glucose levels, higher than 300 mg/dl were used for the experiments and animals were randomized divided into nine groups of six rats.

Groups No. 1 served as diabetic control received distilled water in 5% Tween-80. Groups No. 2 received (positive control) Glibenclamide at an oral dose 10 mg/kg and groups 3-8 received Chloroform leaves and flower extract at the dose of (50, 100, 200 mg/kg) respectively, group No. 9 Normal received distilled water in 5% Tween-80. The treatment were continued daily for 27 days, Blood samples were collected from retro-orbital plexus under anesthesia in centrifuged tube and were centrifuged at 1000 g for 15 min to obtain serum was and used for estimation of glucose using OGENT Glucose kit (Manufactured by Span diagnostic LTD) using star-21plus semi-autoanalyser, after the 1 hrs of treatment on days 1, 7, 14, 21 and 27^{th} days of treatments.

Statistical analysis

Statistical analysis was carried out by student paired and unpaired *t* test Graphpad prism 4.02-version software (USA). All the data were expressed as mean±SEM. Values were considered statistically significant, when ($P<0.05$).

RESULTS AND DISCUSSIONS

In light of the above reports (3), is claimed to be useful for the treatment of diabetes. In order to establish a scientific basis for the utility of this plant in the treatment of diabetes, it was decided to evaluate experimental design of hypoglycemic activity, potentiation action of exogenous insulin, glucose tolerance test and streptozotocin induced diabetes rats. The results of the present study shown in the figure No. I Nyctanthes *arbortristis* leaves and flowers chloroform extract significantly ($P<0.05$, $P<0.01$) decreased fasting blood serum glucose in the normal rats at 1½, 3, 5 hrs as compared to initial blood serum glucose levels (0 hrs). However, the reduction in the blood serum glucose levels is more in flowers extract at a dose of 8 gm/kg, when compared to leaves. The results further revealed that the maximum glucose suppression occurred after 5 hrs of treatment in flowers, was found to be more potential, when compared to leaves extracts. The potentiation action of exogenous insulin of *Nyctanthes arbortristis* leaves and flower chloroform extract at different doses (50, 100, 200 mg/kg) and challenged with Insulin (1 Unit/kg) are presented in the table No. II at 2 hrs of test after 30 mins of extract administration, received Insulin (1 Unit/kg) in all the three different doses of flower and leaves extracts produced significantly ($P<0.05$, $P<0.01$, $P<0.001$) lower serum glucose levels, when compared to the initial glucose levels (0 hrs). A maximum decrease was observed with 100, 200 mg/kg of flowers and leaves extracts. These doses also produced a significant ($P<0.05$, $P<0.01$, $P<0.001$) decrease in serum glucose at 30 mins, 1, 2 hrs, however administration of Insulin alone decreased serum blood glucose significant ($P<0.05$, $P<0.01$), but decrease in serum blood glucose is less, when compared to extract treated along with insulin (1 Unit/kg). These results indicate that extract potentiates exogenous Insulin.

The leaves and flowers chloroform extract at different doses (50, 100, 200 mg/kg) and the positive control on serum glucose levels are challenged with a glucose load are presented in the table No. III at the 180 mins after glucose load, serum glucose levels in all the animals reached a peak at 90-180 mins, the three doses of leaves and flowers extract produced significantly ($P<0.05$, $P<0.01$, $P<0.001$) lower serum glucose levels, compared to the control. A maximum decrease was observed with 100, 200 mg/kg of flower extract. These doses also produced a significant decrease in serum glucose at 90, 180 mins. However the extract (50 mg/kg) of leaves did not produce any significant ($P>0.05$) decrease in serum glucose levels in these rats, when compared to the control, the results are not shown in the table. The reference drugs glibenclamide at an oral dose (10 mg/kg) caused a significantly ($P<0.001$) decrease in serum glucose levels at 30, 90 and 180 mins compared to with control.

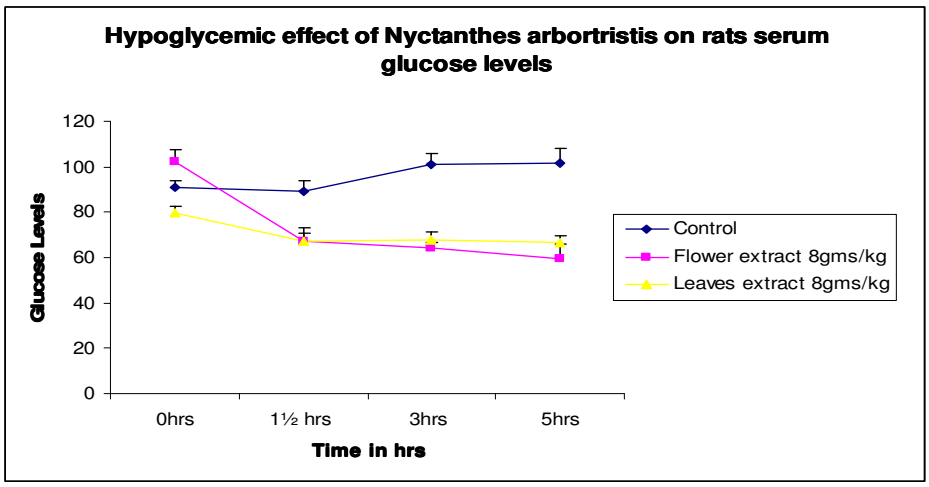

Fig. I: Hypoglycemic effect of Nycatanthes arbortristis on serum glucose levels

Table II: Potentiation of the action of exogenous Insulin of Nyctanthes arbortristis on rat serum glucose

Treatment	Blood Withdrawal at the time of intervals			
	0 hrs (mg/dl)	30 min (mg/dl)	1 hrs (mg/dl)	2 hrs (mg/dl)
Control (Tween-80)	87.55±3.342	87.05 ±4.108	85.7±1.627	80.38±2.395
Insulin (1 Unit/kg)	71.23±5.301	55.24 ±3.882*	54.21±4.778*	48.48±3.575**
Flowers extracts 50 mg/kg + Insulin (1 Unit/kg)	75.05±2.367	62.55±4.038**	50.05±3.909***	47.55±3.899***
Flowers extracts 100 mg/kg +Insulin (1 Unit/kg)	82.05±3.540	66.73±5.724 **	62.56±3.761***	61.72±3.033***
Flowers extracts 200 mg/kg + Insulin (1 Unit/kg)	76.04±5.071	57.69±6.308**	45.54±3.402***	39.71±2.204***
Leaves extract 50 mg/kg + Insulin (1 Unit/kg)	76.04±5.071	57.69±6.308**	52.89±6.291**	46.26±4.740***
Leaves extract 100 mg/kg +Insulin (1 Unit/kg)	64.23±5.386	55.04±4.607*	44.89±2.582**	40.72±2.758**
Leaves extract 200 mg/kg +Insulin (1 Unit/kg)	74.38±4.784	57.38±4.972***	51.38 ±3.101***	50.82±3.858***

*Values are mean ± SEM, n=6 in each group *P < 0.05), **P < 0.01 ***P < 0.001, when 0 hrs (paired t test) compared with 30 min, 1, 2 hrs.*

Table III: Glucose Tolerance Test of Nyctanthes arbortristis on rat serum glucose

Treatment	Blood Withdrawal at the time of intervals			
	0 hrs (mg/dl)	30 min (mg/dl)	90 min (mg/dl)	180 min (mg/dl)
Control (Glucose 2 gm/kg)	78.20±3.496	153.7±4.604	144.1±3.932	122.7± 3.101
Glibencliamide 10 mg/kg	75.4±2.163	116.9±5.369***	94.56±3.126***	78.73±4.038***
Flowers extracts 50 mg/kg + Glucose 2g/kg	69.91±2.94	124.1± 8.505*	109.2± 8.424**	94.62±3.82***
Flowers extracts 100 mg/kg +Glucose 2g/kg	86.63±3.594	137.2±2.205**	95.25 ±9.662***	93.76 ±2.165***
Flowers extracts 200 mg/kg + Glucose 2g/kg	68.82 ±1.213	125.00±6.92**	95.13± 3.753***	90.27± 2.306***
Leaves extract 100 mg/kg + Glucose 2g/kg	81.38±21.29	138.7±2.811*	122.7±3.113**	106.7± 3.415**
Leaves extract 200 mg/kg + Glucose 2g/kg	76.72±3.212	125.4±2.280***	121.4±1.828 ***	108.0 ±4.033*

*Values are mean ± SEM, n=6 in each group *P < 0.05), **P < 0.01 ***P < 0.001, when (Unpaired t test) Compared to control.*

Table IV: Effect Nyctanthes arbortristis on Streptozotocin induced diabetes rat serum glucose

Treatment	Serum glucose (mg/dl) Days				
	1	7	14	21	27
Control: STZ (Tween-80)	348.3±13.44	346.8±12.66	351.5±11.96	346.1±11.23	352.0±9.064
Glibenc10 mg/kg	197.1±10.66***	210.6±8.399***	176.0±9.404***	192.4±5.753***	160.0±8.684***
NFCH 50mg/kg	264.2±15.01**	243.6±22.36**	233.9±15.12***	226.4±17.19***	228.1±13.08***
NFCH 100mg/kg	289.9±5.521**	283.0±7.838**	282.4±12.22**	286.0±9.372 **	273.0±8.859***
NFCH 200mg/kg	300.4±6.051**	290.7±8.147**	288.5±14.39**	292.2±6.349**	271.7±9.944***
NLCH 50mg/kg	276.7±13.64**	281.2±9.741**	271.8±17.48**	277.4±6.144***	266.6±9.388***
NLCH 100mg/kg	275.1±12.71**	281.6±9.047**	293.7±5.949**	296.6±4.051**	315.2±4.830**
NLCH 200mg/kg	284.7±11.29 **	297.7±6.169**	298.2±3.754 **	293.5±2.799**	312.5±4.239**
Normal	74.99±6.747	75.33±4.183	67.02±2.349	76.20±5.675	71.50±4.370

*Control: STZ (Tween-80); Gliben: Glibenclamide; NFCH: Nyctanthes arbortristis flowers; NLCH: Nyctanthes arbortristis leaves; chloroform extracts. Values are mean ± SEM, n=6 in each group *P < 0.05), **P < 0.01 ***P < 0.001, when (Unpaired t test) Compared to control.*

The streptozotocin induced diabetes rats up to 27 day of *Nyctanthes arbortristis* leaves and flowers chloroform extract oral administration produced significant decrease in serum glucose levels in streptozotocin induced diabetic rats. The results are presented in Table. No. IV. *Nyctanthes arbortristis* leaves and flower chloroform extract significantly (P<0.05, P<0.01, P<0.001) decreased fasting blood serum glucose in the streptozotocin treated rats at 1, 7, 14, 21 and 27th day of treatment, when compared to control. However, the reduction in the blood serum glucose levels is more significant (P<0.001), at 14, 21 and 27th in flower extract at a dose of 50 mg/kg, when compared other doses. The results further revealed that the maximum glucose suppression occurred at 27th day of treatment in flower, found to be more potential, when compared to leaves extracts. On comparison, the decrease in blood glucose levels was found to be more pronounced in the flower extracts. The reference drugs Glibenclamide at an oral dose (10 mg/kg) caused a significantly (P<0.001) decreased in serum glucose levels at 1, 7, 14, 21 and 27th days of treatment which is compared with control.

The *Nyctanthes arbortristis* found relative higher LD_{50} 16g/kg (21) of extract probably suggest that the plant extract is safe in rats. The main classes of synthetic oral hypoglycaemic agents currently available for the managements or control of adults-onset type 2 Non-Insulin-Dependent Mellitus (NIDDM), include the sulphonylureas, biguanides, thazolidinediones, alpha-glucosidase inhibitor, as a class, sulphonylureas stimulate and increase the release of endogenous Insulin from pancreatic ß-cell. The *Nyctanthes Arbortristis* plant extracts was reported, analgesic, anti-inflammatory, *In vitro* and *In vivo* anti-trypanosomal, tranquiling, antihistamine and purgative properties in the laboratory animals model (4, 6, 7, 21) the antitrypanosomal activity (7), either due to the presence of iridoid glucosides, mainly ß-Sitosterol, 6ß-Hydroxyloganin which as active constitutes against *plasmodium spp* and *lishmania spp*. So the chloroform extract used in this study caused significant reduction in the blood glucose levels of the fasted normal and in streptozotocin induced diabetic rats. The mechanism of the hypoglycaemic effect of the plants extract is unknown at the moments. *Nyctanthes arbortristis* has been reported to contain iridoid glucosides, mainly ß-Sitosterol, 6-ßHydroxyloganin 1 and 2 from leaves (5, 22). The ß-Sitosterol is unlikely to account for blood glucose lowering action of *Nyctanthes arbortristis*. At present, the may be chemical constitutes of *Nyctanthes arbortristis* are responsible for the observed blood glucose lowering effect. However, a number of investigators have shown that a host of secondary plant metabolites with diverse chemical structures possess the latter properties in various experimental animals model (5, 22).

Since *Nyctanthes arbortristis* are known to contain large quantities of ß-Sitosterol, 6-ßHydroxyloganin, it's not unreasonable to speculate that these chemical compounds might have contributed at least in part to the observed decreased in blood serum glucose effect of extract in this study (5, 22). Thus folk's use of this plant may be validated by this study; however, controlled clinical trial will be required to confirm its activity and general safety.

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Phcog Mag. Vol 4, Issue 16, Oct-Dec, 2008

Submitted on : 25th January, 2008
Revised on : : 11th March, 2008
Accepted on: : 22nd May, 2008

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