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 glibenclamide 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

**INTRODUCTION**

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 *Nytanthes arboristris* of different extracts on normal and streptozotocin induced diabetic rats. *Nytanthes arboristris* 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 histolytica* (7), anti-inflammatory and antioxidant activity (8, 9). The isolated arboritostosides-A from the ethanolic extract of its seeds, shown anti-inflammatory and analgesic activity (10). *Nytanthes arboristris* have shown pro and anti-inflammatory cytokines by water-soluble ethanol extracts (11). Two pure compounds isolated from the plant *Nytanthes arboristris* were tested against Encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV) (12). *Nytanthes arboristris* 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 (arboritostosides-A [I], B [2], C [3], and 6beta-hydroxyloganin [4] shown antileishmanial activity in both in vitro and in vivo test systems (14). The phytochemical analysis of leaves of *Nytanthes arboristris* reveals the presence of beta-amyrin, beta-sitosterol, hentriacontane, benzoic acid, glycosides, nytanthoside-a iridoid, nytanchic acid, friedlin leupeol and oleanonic acid and 6β-hydroxyloganin iridoid glucosides-arborsides A, B and C (12).

**MATERIAL AND METHOD**

*Nytanthes arboristris* 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°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. J819/95, 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 *Nytanthes arboristris* flower and leaves chloroform extract at dose of 8 gm/kg respectively, the dose administered after 24hr 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 collected 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 Gilbencamide 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 extract 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-autoanlyser.

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 intraperitoneally. 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 27th 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. 1 Nyctanthes arbor-tristis 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 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 arbor-tristis 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 (R<0.001) decrease in serum glucose levels at 30, 90 and 180 mins compared to control.
Hypoglycemic effect of Nyctanthes arboristris on rats serum glucose levels

Fig. 1: Hypoglycemic effect of Nyctanthes arboristris on serum glucose levels

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood Withdrawal at the time of intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hrs (mg/dl)</td>
</tr>
<tr>
<td>Control (TWEEN-80)</td>
<td>87.55±3.342</td>
</tr>
<tr>
<td>Insulin 1 Unit/kg</td>
<td>71.23±5.301</td>
</tr>
<tr>
<td>Flowers extracts 50 mg/kg + Insulin 1 Unit/kg</td>
<td>75.05±2.367</td>
</tr>
<tr>
<td>Flowers extracts 100 mg/kg + Insulin 1 Unit/kg</td>
<td>82.05±3.540</td>
</tr>
<tr>
<td>Flowers extracts 200 mg/kg + Insulin 1 Unit/kg</td>
<td>76.04±5.071</td>
</tr>
<tr>
<td>Leaves extract 50 mg/kg + Insulin 1 Unit/kg</td>
<td>76.04±5.071</td>
</tr>
<tr>
<td>Leaves extract 100 mg/kg + Insulin 1 Unit/kg</td>
<td>64.23±5.386</td>
</tr>
<tr>
<td>Leaves extract 200 mg/kg + Insulin 1 Unit/kg</td>
<td>74.38±4.784</td>
</tr>
</tbody>
</table>

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 arboristris on rat serum glucose

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood Withdrawal at the time of intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hrs (mg/dl)</td>
</tr>
<tr>
<td>Control (Glucose 2 gm/kg)</td>
<td>78.20±3.496</td>
</tr>
<tr>
<td>Glibenclamide 10 mg/kg</td>
<td>75.4±2.163</td>
</tr>
<tr>
<td>Flowers extracts 50 mg/kg + Glucose 2g/kg</td>
<td>69.9±2.94</td>
</tr>
<tr>
<td>Flowers extracts 100 mg/kg +Glucose 2g/kg</td>
<td>86.6±3.594</td>
</tr>
<tr>
<td>Flowers extracts 200 mg/kg +Glucose 2g/kg</td>
<td>68.8±1.213</td>
</tr>
<tr>
<td>Leaves extract 100 mg/kg + Glucose 2g/kg</td>
<td>81.3±2.129</td>
</tr>
<tr>
<td>Leaves extract 200 mg/kg + Glucose 2g/kg</td>
<td>76.7±3.212</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Treatment</th>
<th>Serum glucose (mg/dl)</th>
<th>Days</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control: STZ (Tween-80)</td>
<td>348.3±13.44</td>
<td></td>
<td>346.8±17.66</td>
<td>351.5±11.96</td>
<td>346.1±11.23</td>
<td>352.0±49.064</td>
<td></td>
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<tr>
<td>Glibenclamide 10 mg/kg</td>
<td>197.1±10.66***</td>
<td></td>
<td>210.6±8.399***</td>
<td>176.0±9.404***</td>
<td>192.4±5.753***</td>
<td>160.0±8.684***</td>
<td></td>
</tr>
<tr>
<td>NFCH 50 mg/kg</td>
<td>264.2±15.01**</td>
<td></td>
<td>243.6±22.36**</td>
<td>235.9±15.12***</td>
<td>226.4±17.19***</td>
<td>228.1±13.08***</td>
<td></td>
</tr>
<tr>
<td>NFCH 100 mg/kg</td>
<td>289.9±5.521**</td>
<td></td>
<td>238.0±7.838**</td>
<td>282.4±12.22**</td>
<td>286.0±9.372**</td>
<td>273.0±8.859**</td>
<td></td>
</tr>
<tr>
<td>NFCH 200 mg/kg</td>
<td>300.4±6.051**</td>
<td></td>
<td>290.7±8.147**</td>
<td>288.5±14.39**</td>
<td>292.2±6.349**</td>
<td>271.7±9.944**</td>
<td></td>
</tr>
<tr>
<td>NLCH 50 mg/kg</td>
<td>276.7±13.64**</td>
<td></td>
<td>281.2±9.741**</td>
<td>271.8±17.48**</td>
<td>277.4±6.144**</td>
<td>266.6±9.388***</td>
<td></td>
</tr>
<tr>
<td>NLCH 100 mg/kg</td>
<td>275.1±12.71**</td>
<td></td>
<td>281.6±9.047**</td>
<td>293.7±5.949**</td>
<td>296.6±4.051**</td>
<td>315.2±4.830**</td>
<td></td>
</tr>
<tr>
<td>NLCH 200 mg/kg</td>
<td>284.7±11.29**</td>
<td></td>
<td>297.7±6.169**</td>
<td>298.2±3.754**</td>
<td>293.5±2.799**</td>
<td>312.5±4.239**</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>74.99±6.747</td>
<td></td>
<td>75.3±4.183</td>
<td>67.0±2.349</td>
<td>76.20±5.675</td>
<td>71.50±4.370</td>
<td></td>
</tr>
</tbody>
</table>

Control: STZ (Tween-80); Glibenclamide; NFCH: Nychanthes arboritris flowers; NLCH: Nychanthes arboritris 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 Nychanthes arboritris 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. IV. Nychanthes arboritris 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 27 th days of treatment which is compared with control.

The Nychanthes arboritris found relative higher LĐ50 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 B-cell. The Nychanthes Arboiritris plant extracts was reported, analgesic, anti-inflammatory, in vitro and in vivo anti-trypanosomal, tranquilizing, antihistamine and purgative properties in the laboratory animals model (4, 6, 7, 21) the anti-trypanosomal activity (7), either due to the presence of tridiod glucosides, mainly B-Sitosterol, 6β-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. Nychanthes arboritris has been reported to contain iridoid glucosides, mainly B-Sitosterol, 6β-Hydroxyloganin 1 and 2 from leaves (5, 22). The B-Sitosterol is unlikely to account for blood glucose lowering action of Nychanthes arboritris. At present, the may be chemical constitutes of Nychanthes arboritris 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 Nychanthes arboritris are known to contain large quantities of B-Sitosterol, 6β-Hydroxyloganin, 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.

References


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Prevention of high-fructose diet induced Insulin resistance by
*Nyctanthes arbor-tristis* and *Calotropis gigantea* in rats

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Abstract
We have investigated the effect of *Nyctanthes arbor-tristis* (50, 100, 200 mg/kg) and *Calotropis gigantea* leaves and flower chloroform (10, 20, 50 mg/kg) and *Calotropis gigantea* flower petroleum ether extracts (10, 20, 50 mg/kg) in high-fructose diet induced insulin resistance in rats. The fasting serum glucose, insulin, triglyceride and cholesterol levels were measured in blood serum for 27 days of treatment. The fasting serum glucose, insulin, insulin resistance index (FIRI) levels of high-fructose diet (control) rats significantly (P<0.001 vs. normal) increased, like wise, serum triglyceride, cholesterol levels significantly (P=0.001-P<0.01 vs. normal) increased. The *Nyctanthes arbor-tristis* and *Calotropis gigantea* leaves and flower treatment prevent significantly (P<0.001-P<0.01) vs. control) increase serum glucose, insulin, levels in high fructose-diet treated rats, except in glucose *Calotropis gigantea* leaves 50 mg/kg, while significantly (P=0.05-P<0.01 vs. control) decreased in triglyceride, cholesterol, except in triglyceride *Nyctanthes arbor-tristis* leaves 50 mg and in cholesterol *Nyctanthes arbor-tristis* leaves and flowers 50 mg. Further more, high-fructose diet (control) had higher in FIRI (P<0.001) than normal. In contrast, *Nyctanthes arbor-tristis* and *Calotropis gigantea* significantly (P<0.001) decreased FIRI in the high-fructose diet treated rats.

Key words: *Calotropis gigantea*, Cholesterol, FIRI, Insulin resistance, *Nyctanthes arbor-tristis*, Triglyceride.

Introduction
Over the past decade, per capita consumption of high-fructose corn syrups has increased dramatically. Several author suggested that increased fructose ingestion may be responsible for the epidemic of obesity and the increased incidence of metabolic syndrome and diabetic (1). Diets rich particularly fructose, have been shown to be associated with hypertriglyceridemia both in human and rodents (2, 3). Fructose fed rats were shown to have an impaired ability to suppress hepatic glucose production and to eliminate peripheral glucose. The increased in the gluconeogenic enzymes glucose-6-phosphatase and phosphoenol pyruvate, carboxy-kinase in liver fructose fed rats. The fructose induced insulin resistance was associated with a slight decrease in insulin receptor substrate-1/Phosphorylation and insulin receptor Substrate-1/Phosphoinositol 3-kinase associated with the liver and muscles of intact rats (4). The significantly increased in fasting serum glucose, insulin and serum concentration in rats that consumed 15% of energy of fructose (5).

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 (6). Recently, there has been increasing interest in the use of medicinal plants. 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 (7).

*Nyctanthes arbor-tristis* Linn. (Family: *Olaceae*) 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 (8-11). The leaves have also been found to exhibit activity against Plasmodium falciparum, Leishmania donovani and Entamoeba histolytica (12), anti-inflammatory activity of ethanolic extract of tubular calyx and antioxidant activity of carotenoid from NYctanthes arbor-tristis (13-14), isolated arboristressid-A from the ethanolic extract of its seeds, shown anti-inflammatory and analgesic activity (15). NYctanthes arbor-tristis have shown pro and anti-inflammatory cytokines by water-soluble ethanol extracts (16). Two pure compounds isolated from the plant NYctanthes arbor-tristis were tested against Encephalomyocardiitis virus (EMCV) and Semik forest virus (SVF) (17). NYctanthes arbor-tristis 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 (18). Iridoid glucosides (arboristressid-A [1], B [2], C [3]) and 6β-hydroxyloganin [4] isolated from the traditional plant NYctanthes arbor-tristis show antileishmanial activity in both in vitro and in vivo systems (19). The phytochemical analysis of leaves of NYctanthes arbor-tristis reveals the presence of β-amyrin, β-sitosterol, hemitri-aacetone, benzoic acid, glycosides, nycanthoside-a iridoid, nycanthonic acid, and iridoid glucoside-arborside A, B and C (12).

Caltrops gigantea Linn. (Family: Asclepiadaceae) is a perennial undershrub found chiefly in wastelands throughout India. Traditionally the plant is used as analgesic, cures toothache and earache, sprain, anxiety and pain in epilepsy and in mental disorders; this plant is also reported to possess emmenagogue, uterotonic, psychic and abortifacient activities. Tincture of the leaves of this plant is used for the treatment of intermittent fevers, and the powdered flowers are beneficial in treating colds, coughs, asthma and indigestion (20). It has been reported pregnancy intermittent activity of the ethanolic in roots of Caltrops gigantea (20), anti-diarrheal, analgesic effect of hydroalcoholic (50:50) of extract (21, 22), antipyretic activity of using yeast-induced and TAB (typhoid) vaccine-induced pyrexia (23). The alcoholic extract of peeled roots of Caltrops gigantea observed CNS activity in albino rats, prominent analgesic activity was observed in Eddy's hot plate and acetic acid induced writhing (24). The milky juice of this plant has been reported as a violent purgative and gastrointestinal irritant and has been used for inducing abortion (25). The alcoholic extract of the flowers of Caltrops gigantea analgesic activity in chemical and thermal models in mice (26). Procoagulant activity of Caltrops gigantea latex associated with fibrinogenolytic activity, the latex of Caltrops gigantea in controlling bleeding (27). The constituents isolated are glycosides and proteases, the occurrence of 3'-methylbutanoates of α-amyrin, β-amyrin and γ-taraxasterol from Caltrops gigantea (28). New flavonol trisaccharide was isolated from the aerial parts of Caltrops gigantea and its structure was established as isorhamnetin-3-O-[2-O-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl]-β-D-glucopyranoside by a combination of fast atom bombardment mass spectroscopy, 'H and C' NMR spectra and some chemical degradations (29). Caltrops D1 and DII isolated from the latex of madar plants, Caltrops gigantea, classified as plant, cysteine proteases, papain, ficin and stem bromelain, caltropin D1 is more susceptible to autodigestion than caltropin DII. During autodigestion no interconversion of one caltropin to another has occurred, immunologically, both caltropins are closely related, but they differ from papain and ficin. Both caltropins have blocked N-terminal amino acid residues. Their C-terminal amino acid sequences, determined by treatment with carboxypeptidase Y, are - (Pro, Ala)-Ala-Val-Tyr for caltropin D1 and - (Ala, Val)-Ala-Pro-Tyr for caltropin DII. The tryptic peptide maps of their reduced and S-carboxymethylated derivatives suggest that both caltropins share a high proportion of common regions in their amino acid sequences. Caltrops D1 and DII are two distinct proteases (30). The three-dimensional structure of the sulphhydryl protease caltropin D1 from the madar plant. Caltrops gigantea, has been determined at 3.2A° a resolution using the multiple isomorphous replacement method with five heavy atom derivatives. The overall molecular architecture closely resembles those found in the Sulphydryl proteases papain and actinidin (31).

MATERIAL AND METHOD

NYctanthes arbor-tristis and Caltrops gigantea leaves and flowers were collected from widely growing plants in the region of north Karnatak in the months of Sept.-October 2005. The plant material was dried in shade and coarse powdered and extracted with petroleum ether to (40-60°C) defat, chloroform, 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
Prevention of high-fructose diet induced Insulin resistance by Nyctanthes arbor-tristis and Calotropis gigantea in rats

5% Tween-80, as suspending agent (32). Wistar albino rats (200-250 g) 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. The dose of Nyctanthes arbor-tristis and Calotropis gigantea was selected based on previous study (9-11, 20-24). The qualitative test of the crude extract shows the presence of alkaloids, and flavon glycoside supporting the earlier studies.

Assay: - Serum glucose, triglyceride and cholesterol determination were performed using OGEN kit (Manufactured by Span diagnostic LTD) by using a star-21plus semi-autoanlyser, insulin levels was determined by using radioimmunassay technique (Board of Radiation and Isotope Technology Mumbia). Consignment No: 0802003).

Animals preparation (4, 56, 33)
Insulin resistance was induced in the rats by high-fructose diet containing fructose-624 g/kg, fats as vegetable oils 5 g/kg, protein 223 g/kg, necessary amino acids, vitamins 1.2% and minerals, normal rats was fed with standard laboratory chow, at the beginning of the experiments the animals were divided into 17 groups of six rats groups. Group 1 served as normal, group 2 control (high-fructose diet). received 0.5 ml of 5% Tween 80, groups 3-8 received high-fructose diet-Nyctanthes arbor-tristis leaves and flower of chloroform at the dose (50, 100, 200 mg/kg), groups 9-14, received high fructose-diet Calotropis gigantea leaves and flower chloroform (10, 20, 50 mg/kg) and group 15-17 received high-fructose diet-Calotropis gigantea flower (10, 20, 50 mg/kg) of petroleum extracts. The treatment was continued for 27 days. At the end of the treatment periods (after an over night fasting) blood samples were collected from retro-orbital plexus under anesthesia, were centrifuged at 1000 rpm for 15 min to obtain serum was used for estimation of serum glucose, insulin, triglyceride and cholesterol levels.

Insulin Resistance Calculation:
Fasting insulin resistance index (FIRI) were calculated according to the formula (3, 6)

\[
FIRI = \frac{\text{Fasting insulin} \times \text{fasting glucose}}{25}
\]

Statistical data analysis
Statistical analysis was carried out by student (unpaired t test) using Graphpad prism 4.02-version software (USA). All the data were expressed as mean ± SEM.

RESULTS
The effect of Nyctanthes arbor-tristis (50, 100, 200 mg/kg) and Calotropis gigantea leaves and flower chloroform (10, 20, 50 mg/kg) and Calotropis gigantea flower petroleum ether extracts (10, 20, 50 mg/kg) in high-fructose diet induced insulin resistance in rats. The fasting serum glucose, insulin (μU/ml), triglyceride and cholesterol levels were measured in blood serum for 27 days of treatment groups of rats have shown in table (1 and 2). The fasting serum glucose, insulin, insulin resistance index (FIRI) levels of high-fructose diet (control) rats significantly (P<0.001 vs. normal) increased, like wise, serum triglyceride, cholesterol significantly (P<0.001-P<0.01 vs. normal) increased. The Nyctanthes arbor-tristis and Calotropis gigantea leaves and flower treatment prevent significantly (P<0.001-P<0.01) vs. control) increase serum glucose, insulin, levels in high-fructose diet treated rats, except in glucose Calotropis gigantea leaves 50 mg/kg, also significantly (P<0.05-P<0.01 vs. control) decreased in triglyceride, cholesterol, except in triglyceride Nyctanthes arbor-tristis leaves 50 mg and in cholesterol Nyctanthes arbor-tristis leaves and flower 50 mg. Further more, high-fructose diet (control) had higher in FIRI (P<0.001) than normal. In contrast, Nyctanthes arbor-tristis and Calotropis gigantea significantly (P<0.001) decreased FIRI in the high-fructose diet treated rats. These results indicate that administration of low doses of treated rats may be advantageous for preservation of the functional characteristics of pancreatic beta cells, probably by improving insulin action and thereby insulin resistance prevention.

DISCUSSION
Insulin resistance in human has been shown to be present in conditions like NIDDM, Obesity and dyslipidemia, thus intervention to decrease insulin resistance may post pone the development of NIDDM and its complication (6). The present study indicates that fructose induced hypertriglyceridemia is associated with significant hyperinsulinemia. The high-fructose diet stimulates the hepatic production of triglyceride, both by promoting the reesterification of circulating non-esterified fatty acids and by stimulating de Novo fatty acids synthesis increased delivery of triglyceride or non-esterified fatty acids to the muscles interfere with the utilization of glucose, through the principles of Randle cycle, impairing the insulin action (3). Fructose fed rats was shown to have an impaired ability to suppress hepatic glucose production and to eliminate peripheral glucose. Fructose fed shown significantly increased in fasting
Table 1: Effect of N. arbortristis and C. gigantea on glucose, triglyceride and cholesterol in HFDI insulin resistance in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>97.80 ± 3.802</td>
<td>115.1 ± 6.828</td>
<td>90.85 ± 4.405</td>
</tr>
<tr>
<td>Control (Diet)</td>
<td>148.8 ± 3.865***</td>
<td>167.8 ± 10.89**</td>
<td>123.1 ± 3.819***</td>
</tr>
<tr>
<td>FD-NFCH 100 mg/kg</td>
<td>97.11 ± 7.414***</td>
<td>136.2 ± 4.725*</td>
<td>108.6 ± 5.328ns</td>
</tr>
<tr>
<td>FD-NFCH 200 mg/kg</td>
<td>99.86 ± 5.324***</td>
<td>121.2 ± 1.213**</td>
<td>101.8 ± 2.910**</td>
</tr>
<tr>
<td>FD-NLCH 50 mg/kg</td>
<td>100.2 ± 5.482***</td>
<td>122.0 ± 2.207**</td>
<td>98.45 ± 3.827**</td>
</tr>
<tr>
<td>FD-NLCH 100 mg/kg</td>
<td>94.34 ± 6.518***</td>
<td>145.8 ± 3.068ns</td>
<td>112.9 ± 5.212ns</td>
</tr>
<tr>
<td>FD-NLCH 200 mg/kg</td>
<td>101.2 ± 7.957***</td>
<td>133.3 ± 2.816*</td>
<td>102.3 ± 4.231**</td>
</tr>
<tr>
<td>FD-NLCH 200 mg/kg</td>
<td>100.4 ± 7.757***</td>
<td>123.3 ± 2.977**</td>
<td>97.55 ± 4.130**</td>
</tr>
<tr>
<td>ED-CFCH 10 mg/kg</td>
<td>132.1 ± 2.007**</td>
<td>136.5 ± 1.853*</td>
<td>101.6 ± 4.057**</td>
</tr>
<tr>
<td>ED-CFCH 20 mg/kg</td>
<td>137.0 ± 1.698</td>
<td>134.5 ± 3.443*</td>
<td>96.90 ± 4.702**</td>
</tr>
<tr>
<td>ED-CFCH 50 mg/kg</td>
<td>132.4 ± 2.975</td>
<td>139.6 ± 3.687*</td>
<td>97.02 ± 6.261**</td>
</tr>
<tr>
<td>ED-NLCH 10 mg/kg</td>
<td>114.5 ± 4.853***</td>
<td>121.5 ± 5.514**</td>
<td>100.9 ± 5.351**</td>
</tr>
<tr>
<td>ED-NLCH 20 mg/kg</td>
<td>119.4 ± 8.139**</td>
<td>140.9 ± 4.520*</td>
<td>102.2 ± 4.305**</td>
</tr>
<tr>
<td>ED-NLCH 50 mg/kg</td>
<td>141.9 ± 3.500ns</td>
<td>137.0 ± 2.138*</td>
<td>106.9 ± 3.877*</td>
</tr>
<tr>
<td>ED-CFPE 10 mg/kg</td>
<td>109.0 ± 7.519***</td>
<td>125.3 ± 1.546**</td>
<td>108.4 ± 3.549*</td>
</tr>
<tr>
<td>ED-CFPE 20 mg/kg</td>
<td>109.7 ± 6.307***</td>
<td>12.7 ± 4.166**</td>
<td>109.0 ± 3.589*</td>
</tr>
<tr>
<td>ED-CFPE 50 mg/kg</td>
<td>117.2 ± 5.068***</td>
<td>131.1 ± 3.127**</td>
<td>101.4 ± 4.702**</td>
</tr>
</tbody>
</table>

All the data were expressed as mean ± SEM by using student t test (unpaired t test) Values were, compared to control *P <0.05), **P <0.01 ***P <0.001. HFDI=high-fructose diet induced; N. arbortristis=Nyctanthes arbortristis; C. gigantea=Calotropis gigantea; FD-NFCH=Fructose-diet-N. arbortristis flowers chloroform; FD-NLCH=Fructose-diet-N. arbortristis leaves chloroform; ED-CFCH=Fructose-diet-C. gigantea flowers chloroform; ED-CFPE=Fructose-diet-C. gigantea leaves chloroform.

Table 2: Effect of N. arbortristis and C. gigantea on insulin, FIRI in HFDI insulin resistance in rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Insulin µU/ml</th>
<th>FIRI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>36.33 ± 1.801</td>
<td>144.6 ± 9.45</td>
</tr>
<tr>
<td>Control (fructose Diet)</td>
<td>87.17 ± 6.949***</td>
<td>522.2 ± 50.14 ***</td>
</tr>
<tr>
<td>FD-NFCH 50 mg/kg</td>
<td>37.50 ± 4.552***</td>
<td>155.0 ± 28.33 ***</td>
</tr>
<tr>
<td>FD-NFCH 100 mg/kg</td>
<td>39.33 ± 5.312***</td>
<td>160.5 ± 22.08 ***</td>
</tr>
<tr>
<td>FD-NFCH 200 mg/kg</td>
<td>39.00 ± 4.443***</td>
<td>155.0 ± 17.06 ***</td>
</tr>
<tr>
<td>FD-NLCH 50 mg/kg</td>
<td>38.83 ± 3.188***</td>
<td>145.1 ± 11.17 ***</td>
</tr>
<tr>
<td>FD-NLCH 100 mg/kg</td>
<td>44.00 ± 6.676***</td>
<td>172.2 ± 10.12 ***</td>
</tr>
<tr>
<td>FD-NLCH 200 mg/kg</td>
<td>47.83 ± 3.440***</td>
<td>189.0 ± 14.06 ***</td>
</tr>
<tr>
<td>ED-CFCH 10 mg/kg</td>
<td>39.67 ± 2.028***</td>
<td>209.7 ± 13.05 ***</td>
</tr>
<tr>
<td>ED-CFCH 20 mg/kg</td>
<td>43.67 ± 0.615***</td>
<td>244.0 ± 2.777 ***</td>
</tr>
<tr>
<td>ED-CFCH 50 mg/kg</td>
<td>53.67 ± 4.072**</td>
<td>281.7 ± 20.32 **</td>
</tr>
<tr>
<td>ED-CLCH 10 mg/kg</td>
<td>38.50 ± 1.607***</td>
<td>174.5 ± 9.722 ***</td>
</tr>
<tr>
<td>ED-CLCH 20 mg/kg</td>
<td>38.33 ± 1.585***</td>
<td>183.6 ± 7.367 ***</td>
</tr>
<tr>
<td>ED-CLCH 50 mg/kg</td>
<td>63.33 ± 3.761*</td>
<td>361.2 ± 43.06*</td>
</tr>
<tr>
<td>ED-CFPE 10 mg/kg</td>
<td>37.67 ± 0.843***</td>
<td>163.9 ± 10.19 ***</td>
</tr>
<tr>
<td>ED-CFPE 20 mg/kg</td>
<td>40.67 ± 1.838***</td>
<td>165.4 ± 14.18 ***</td>
</tr>
<tr>
<td>ED-CFPE 50 mg/kg</td>
<td>41.17 ± 5.890***</td>
<td>194.9 ± 33.75 ***</td>
</tr>
</tbody>
</table>

All the data were expressed as mean ± SEM by using student t test (unpaired t test) when compared to control *P <0.05, **P <0.01 ***P <0.001. FIRI=Fasting insulin resistance index; HFDI=high-fructose diet induced; N. arbortristis=Nyctanthes arbortristis; C. gigantea=Calotropis gigantea; FD-NFCH=Fructose diet-N. arbortristis flowers chloroform; FD-NLCH=Fructose-diet-N. arbortristis leaves chloroform; ED-CFCH=Fructose-diet-C. gigantea flowers chloroform; ED-CFPE=Fructose-diet-C. gigantea leaves chloroform.
Prevention of high-fructose diet induced insulin resistance by Nycanthes arbortristis and Calotropis gigantea in rats

serum glucose and insulin concentration in rats that consumed 15% of energy of fructose (5). Administration of Nycanthes arbortristis and Calotropis gigantea at different dose for 27 days in high-fructose diet rats significantly decreased serum glucose, insulin, triglyceride, cholesterol, and FINS, compared with the control. Therefore, the available information strongly supports the close interrelationship between insulin resistance and hypertriglyceridemia (3). Treatment with natural herbls like, Nycanthes arbortristis and Calotropis gigantea with lesser side effect compared to the presently used synthetic oral antidiabetic agents. Among the various constituents of Nycanthes arbortristis and Calotropis gigantea extract has been reported to possess various activities. The Nycanthes arbortristis plant extracts was reported, analgesic, anti-inflammatory, in vitro and in vivo antityranosomal, immunostimulant, tranquilizing, antihistamine and purgative in prerties in the mammalian laboratory animals model (9, 11), the antityranosomal activity (12), either due to the presence of iridoid glycosides, mainly B-sitosterol, 68-hydroxyloganin which as active constituent against plasmodium spp and lishmania spp. So the chlorofrom extract used in this study caused significant reduction in the blood serum glucose insulin, triglyceride and cholesterol in fasted rats. The mechanism of these effects of the plants extract is unknown at the moments. Nycanthes arbortristis has been reported to containing iridoid glycosides, namely B-Sitosterol, 68-hydroxyloganin 1 and 2 from leaves (17). The B-sitosterol is unlikely to account for lowering action above parameter. At present, the exact chemical constituent of Nycanthes arbortristis responsible for the above observed effect are still obscure, 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 (10, 19).

Since Nycanthes arbortristis are known to contain large quantities of B-sitosterol, 68-hydroxyloganin, it’s not unreasonable to speculate that these chemical compounds might have contributed at least in part to the observed decrease in blood action serum glucose effect of extract in this study (10, 12). It has been reported pregnancy interpetative activity of the roots of Calotropis gigantea Linn in rats, the milky juice of this plant has been reported as a violent purgative and gastrointestinal irritant and has been used for inducing abortion (20), anti-diarrhelal, analgesic effect of hydroalcoholic (50:50) of extract (21-22), antipyretic activity of using yeast-induced and TAB (typhoid) vaccine-induced pyrexia (23). The alcoholic extract of peeled roots of Calotropis gigantea observed CNS activity in albino rats, prominent analgesic activity was observed in Eddy’s hot plate and acetic acid induced writhings (24). The alcoholic extract of the flower of Calotropis gigantea analgesic activity in chemical and thermal models in mice (25-26). Procoagulant activity of Calotropis gigantea latex associated with fibrin(ogen)olytic activity, the latex of Calotropis gigantea in controlling bleeding (27). Previously isolated classes of constituent of Calotropis gigantea is a rich source of several biological molecules, free amino acid, peptides and enzymes and non-enzyme proteins, among others. The constituents isolated are glycosides and proteases, the occurrence of 3-methylbutanoates of α-amyrin, β-amyrin and peta-taxasterol from Calotropis gigantea (28). New flavonol trisaccharide was isolated from the aerial parts of Calotropis gigantea and its structure was established as isorhamnetin-3-O-[2-O-β-D-galactopyranosyl-6-O-α-L-rhamnopyranosyl]-β-D-glucopyranoside by a combination of fast atom bombardment mass spectroscopy, 1H and 13C NMR spectra and some chemical degradations (29). Calotropins Dl and Dll isolated from the latex of madar plants (27), might have contributed at least in part to the observed decreased in blood serum glucose, insulin, triglyceride, cholesterol, in high-fructose diet induced insulin resistance rats. These findings demonstrated that, enhancement of the sensitivity of target tissue to circulating insulin by Nycanthes arbortristis and Calotropis gigantea might be related to lowering above parameter, however, the cellular mechanism by which these effects are mediated are unclear. Therefore our data demonstrate that the improvement of physiological insulin action through enhanced insulin sensitivity in peripheral tissue, as was evident from the decreased glucose and insulin, increased liver and skeletal muscular glycogen stress (6). The extract ameliorating hyperinsulinemia are likely to have greater therapeutic potential as they may also exert beneficial effect on the clinical use of NIDDM, hypertension and coronary artery disease condition.

Conclusion

In conclusion, oral administration of Nycanthes arbortristis and Calotropis gigantea at dose of (50, 100, 200 mg/kg) and (10, 20, 50 mg/kg) respectively lowers serum glucose, insulin, triglyceride, cholesterol, in high-fructose diet. However, further
studies are needed to establish the safety and effectiveness of *Nyctanthes arbor-tristis* and *Calotropis gigantea*.

REFERENCES


Prevention of high-fructose diet induced Insulin resistance by Nyctanthes arbor-tristis and Calotropis gigantea in rats


