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

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ABSTRACT

We have investigated the effect of *Nyctanthes arbortristis* (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, cholesterols significantly (P<0.001-P<0.01 vs. normal) increased. The *Nyctanthes arbortristis* 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 arbortristis* leaves 50 mg and in cholesterol *Nyctanthes arbortristis* leaves and flowers 50 mg. Further more, high-fructose diet (control) had higher in FIRI (P<0.001) than normal. In contrast, *Nyctanthes arbortristis* and *Calotropis gigantea* significantly (P<0.001) decreased FIRI in the high-fructose diet treated rats.

KEYWORDS: *Calotropis gigantea*, Cholesterol, FIRI, Insulin resistance, *Nyctanthes arbortristis*, 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). Fructoses 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/Phosporylation and insulin receptor Substrate-1/Phospoinositol 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 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 (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 arbortristis* (13–14), isolated arbortristoside-A from the ethanolic extract of its seeds, shown anti-inflammatory and analgesic activity (15). *Nyctanthes arbortristis* have shown pro and anti-inflammatory cytokines by water-soluble ethanol extracts (16). Two pure compounds isolated from the plant *Nyctanthes arbortristis* were tested against Encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV) (17). *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 (18). Iridoid glucosides (arbortristosides-A [I], B [2], C [3]) and 6β-hydroxyloganin [4] isolated from the traditional plant *Nyctanthes arbortristis,* show antileishmanial activity in both *In vitro* and *In vivo* systems (19). The phytochemical analysis of leaves of *Nyctanthes arbortristis* reveals the presence of β-amyrin, β-sitosterol, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid, nyctanthic acid, and irioid glucoside-arborside A, B and C (12).

Calotropis 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, epilepsy and in mental disorders; this plant is also reported to possess emmenagogue, uterotonic, ecbolic and abortifacient activities. The 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 interceptive activity of the ethanolic in roots of *Calotropis 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 *Calotropis 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 *Calotropis gigantea* analgesic activity in chemical and thermal models in mice (26). Procoagulant activity of *Calotropis gigantea* latex associated with fibrin(ogen)olytic activity, the latex of *Calotropis gigantea* in controlling bleeding (27). The constituents isolated are glycosides and proteases, the occurrence of 3'-methybutanoates of α-amyrin, β-amyrin and σ-taraxasterol 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-Dglucopyranoside by a combination of fast atom bombardment mass spectroscopy, 'H and C' NMR spectra and some chemical degradations (29). Calotropins DI and DII isolated from the latex of madar plants, *Calotropis gigantea,* classified as plant, cysteine proteases, papain, ficin and stem bromelain, calotropin DI is more susceptible to autodigestion than calotropin DII. During autodigestion no interconversion of one calotropin to another has occurred, Immunologically, both calotropins are closely related, but they differ from papain and ficin. Both calotropins 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 calotropin DI and - (Ala, Val)-Ala-Pro-Tyr for calotropin DII. The tryptic peptide maps of their reduced and S-carboxymethylated derivatives suggest that both calotropins share a high proportion of common regions in their amino acid sequences. Calotropins DI and DII are two distinct proteinases (30). The three-dimensional structure of the sulfhydryl protease calotropin DI from the madar plant. *Calotropis 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 Sulfhydryl proteases papain and actinidin (31).

MATERIAL AND METHOD

Nyctanthes arbortristis and *Calotropis gigantea* leaves and flowers 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, 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 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 arbortristis* 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 flavanol glycoside supporting the earlier studies.

Assay

Serum glucose, triglyceride and cholesterol determination were performed using OGENT kit (Manufactured by Span diagnostic LTD) by using a star-21plus semi-autoanalyser, insulin levels was determined by using radioimmunoassy 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 1.25% 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 arbortristis* 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 colleted 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)

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 arbortristis* (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, cholesterols significantly ($P<0.001$-$P<0.01$ vs. normal) increased.

The *Nyctanthes arbortristis* 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 arbortristis* leaves 50 mg and in cholesterol *Nyctanthes arbortristis* leaves and flower 50 mg. Further more, high-fructose diet (control) had higher in FIRI ($P<0.001$) than normal. In contrast, *Nyctanthes arbortristis* 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 interferes 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 serum glucose and insulin concentration in rats that consumed 15% of energy of fructose (5). Administration of *Nyctanthes arbortristis* and *Calotropis gigantea* at different dose for 27 days in high-fructose diet rats significantly decreased serum glucose, insulin, triglyceride, cholesterol, and FIRI, compared with

the control. Therefore, the available information strongly supports the close interrelationship between insulin resistance and hypertriglyceridemia (3). Treatment with natural herbals like, *Nyctanthes arbortristis* and *Calotropis gigantea* with lesser side effect compared to the presently used synthetic oral antidiabetic agents. Among the various constituents of *Nyctanthes arbortristis* and *Calotropis gigantea* extract has been reported to possess various activities. The *Nyctanthes arbortristis* plant extracts was reported, analgesic, anti-inflammatory, *In vitro* and *In vivo* antitrypanosomal, Immunostimulant, tranquiling, antihistamine and purgative properties in the mammalian laboratory animals model (9, 11), the antitrypanosomal activity (12), either due to the presence of iridoid glycosides, mainly β-sitosterol, 6β-hydroxyloganin which as active constituent against *plasmodium spp* and *lishmania spp*. So the chloroform and petroleum ether 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. *Nyctanthes arbortristis* has been reported to containing iridoid glycosides, mainly β-Sitosterol, 6β-hydroxyloganin 1 and 2 from leaves (17). The β-sitosterol is unlikely to account for lowering action above parameter. At present, the exact chemical constituent of *Nyctanthes 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 *Nyctanthes arbortristis* are known to contain large quantities of β-sitosterol, 6β-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 interceptive 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-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 *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

Table 1: Effect of N. arbortristis and C. gigantea on glucose, triglyceride and cholesterol in HFDI insulin resistance in rats.

Treatment	Glucose (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
Normal	97.80 ± 3.802	115.1 ± 6.828	90.85 ± 4.405
Control (Diet)	148.8 ± 3.865***	167.8 ± 10.89**	123.1 ± 3.819***
FD-NFCH 50 mg/kg	97.11 ± 7.414***	136.2 ± 4.725*	108.6 ± 5.328ns
FD-NFCH 100 mg/kg	99.86 ± 5.324***	121.2 ± 1.213**	101.8 ± 2.910**
FD-NFCH 200 mg/kg	100.2 ± 5.482***	122.0 ± 2.207**	98.45 ± 3.827**
FD-NLCH 50 mg/kg	94.34 ± 5.618***	145.8 ± 3.068ns	112.9 ± 5.212ns
FD-NLCH 100 mg/kg	101.2 ± 7.957***	133.3 ± 2.816*	102.3 ± 4.231**
FD-NLCH 200 mg/kg	100.4 ± 7.757***	123.3 ± 2.977**	97.55 ± 4.130**
FD-CFCH 10 mg/kg	132.1 ± 2.007**	136.5 ± 1.853*	101.6 ± 4.057**
FD-CFCH 20 mg/kg	137.0 ± 1.698 *	134.5 ± 3.443*	96.90 ± 4.702**
FD-CFCH 50 mg/kg	132.4 ± 2.975**	139.6 ± 3.687*	97.02 ± 6.261**
FD-CLCH 10 mg/kg	114.5 ± 4.853***	121.5 ± 5.514**	100.9 ± 5.351**
FD-CLCH 20 mg/kg	119.4 ± 8.139**	140.9 ± 4.520*	102.2 ± 4.505**
FD-CLCH 50 mg/kg	141.9 ± 3.500ns	137.0 ± 2.138*	106.9 ± 3.877*
FD-CFPE 10 mg/kg	109.0 ± 5.189***	125.3 ± 1.546**	108.4 ± 3.549*
FD-CFPE 20 mg/kg	109.7 ± 6.307***	137.8 ± 4.166**	109.0 ± 3.589*
FD-CFPE 50 mg/kg	117.2 ± 5.068***	131.1 ± 3.127**	101.4 ± 4.702**

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; FD-CFCH=Fructose-diet-C. gigantea flowers chloroform; FD-CFCH=Fructose diet-C. gigantea leaves chloroform; FD-CFPE=Fructose-diet-C. gigantea flowers petroleum ether extracts.*

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

Treatment	Insulin µU/ml	FIRI
Normal	36.33 ± 1.801	144.6 ± 9.45
Control (fructose Diet)	87.17 ± 6.949***	522.2 ± 50.14 ***
FD-NFCH 50 mg/kg	37.50 ± 4.552***	155.0 ± 28.33***
FD-NFCH 100 mg/kg	39.33 ± 3.412***	160.5 ± 22.08***
FD-NFCH 200 mg/kg	39.00 ± 4.442***	155.0 ± 17.06***
FD-NLCH 50 mg/kg	38.83 ± 3.188***	145.1 ± 11.17***
FD-NLCH 100 mg/kg	44.00 ± 4.676 ***	172.2 ± 10.12***
FD-NLCH 200 mg/kg	47.83 ± 3.449***	189.0 ± 14.06***
FD-CFCH 10 mg/kg	39.67 ± 2.028***	209.7 ± 13.05***
FD-CFCH 20 mg/kg	43.67 ± 0.615***	244.0 ± 2.777***
FD-CFCH 50 mg/kg	53.67 ± 4.072**	281.7 ± 20.32**
FD-CLCH 10 mg/kg	38.50 ± 1.607***	174.5 ± 9.722***
FD-CLCH 20 mg/kg	38.33 ± 1.585***	183.6 ± 7.367***
FD-CLCH 50 mg/kg	63.33 ± 7.361*	361.2 ± 43.06*
FD-CFPE 10 mg/kg	37.67 ± 0.843***	163.9 ± 10.19***
FD-CFPE 20 mg/kg	40.67 ± 1.838***	165.4 ± 14.18***
FD-CFPE 50 mg/kg	41.17 ± 5.890***	194.9 ± 33.75***

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; FD-CFCH=Fructose-diet-C. gigantea flowers chloroform; FD-CFCH=Fructose-diet-C. gigantea leaves chloroform; FD-CFPE=Fructose-diet-C. gigantea flowers petroleum ether extracts*

non-enzyme proteins, among others. The constituents isolated are glycosides and proteases, the occurrence of 3'-methylbutanoates of α-amyrin, β-amyrin and σ-taraxasterol 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, 'H and C' NMR spectra and some chemical degradations (29). Calotropins DI and DII 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 *Nyctanthes 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 *Nyctanthes 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 arbortristis* and *Calotropis gigantea*.

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