

PHCOG MAG.: Research Article

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

¹Nanu Rathod*, ²I Raghuveer, ³HR Chitme, ⁴Chandra Ramesh

¹*Dept. of Pharmacology, B.V.V Sangh's H.S.K College of Pharmacy, Bagalkot, Karnataka, India

²Institute of Pharmacy, Bundelkhand University, Jhansi, India

³Oman Medical College, West Virginia University, Azaiba, Muscat Sultanate of Oman

⁴Dr. B.R Ambedkar Centre for Biomedical Research, New Delhi, India

*For correspondence : nanurathod@rediffmail.com

ABSTRACT

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

KEYWORDS: *Calotropis gigantea*, Cholesterol, FIRI, Insulin resistance, *Nyctanthes arborescens*, 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 arbortristis Linn. (Family: *Oleaceae*) commonly known as Harsingar 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 arbortristoid-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 (arbortristoides-A [I], B [2], C [3]) and 6 β -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 iridoid 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, ecboic 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 -D-glucopyranoside by a combination of fast atom bombardment mass spectroscopy, ^1H and ^{13}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 3.2Å 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

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

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 radioimmunoassay technique (Board of Radiation and Isotope Technology Mumbai. 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 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)

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 $\pm$ 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 (μ U/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 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 postpone 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 arborescens* and *Calotropis gigantea* with lesser side effect compared to the presently used synthetic oral antidiabetic agents. Among the various constituents of *Nyctanthes arborescens* and *Calotropis gigantea* extract has been reported to possess various activities. The *Nyctanthes arborescens* plant extracts was reported, analgesic, anti-inflammatory, *In vitro* and *In vivo* antitrypanosomal, Immunostimulant, tranquilizing, 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, 6 β -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 arborescens* has been reported to containing iridoid glycosides, mainly β -Sitosterol, 6 β -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 arborescens* 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 arborescens* are known to contain large quantities of β -sitosterol, 6 β -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. arborescens* 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 $\pm$ 3.802	115.1 $\pm$ 6.828	90.85 $\pm$ 4.405
Control (Diet)	148.8 $\pm$ 3.865***	167.8 $\pm$ 10.89**	123.1 $\pm$ 3.819***
FD-NFCH 50 mg/kg	97.11 $\pm$ 7.414***	136.2 $\pm$ 4.725*	108.6 $\pm$ 5.328ns
FD-NFCH 100 mg/kg	99.86 $\pm$ 5.324***	121.2 $\pm$ 1.213**	101.8 $\pm$ 2.910**
FD-NFCH 200 mg/kg	100.2 $\pm$ 5.482***	122.0 $\pm$ 2.207**	98.45 $\pm$ 3.827**
FD-NLCH 50 mg/kg	94.34 $\pm$ 5.618***	145.8 $\pm$ 3.068ns	112.9 $\pm$ 5.212ns
FD-NLCH 100 mg/kg	101.2 $\pm$ 7.957***	133.3 $\pm$ 2.816*	102.3 $\pm$ 4.231**
FD-NLCH 200 mg/kg	100.4 $\pm$ 7.757***	123.3 $\pm$ 2.977**	97.55 $\pm$ 4.130**
FD-CFCH 10 mg/kg	132.1 $\pm$ 2.007**	136.5 $\pm$ 1.853*	101.6 $\pm$ 4.057**
FD-CFCH 20 mg/kg	137.0 $\pm$ 1.698 *	134.5 $\pm$ 3.443*	96.90 $\pm$ 4.702**
FD-CFCH 50 mg/kg	132.4 $\pm$ 2.975**	139.6 $\pm$ 3.687*	97.02 $\pm$ 6.261**
FD-CLCH 10 mg/kg	114.5 $\pm$ 4.853***	121.5 $\pm$ 5.514**	100.9 $\pm$ 5.351**
FD-CLCH 20 mg/kg	119.4 $\pm$ 8.139**	140.9 $\pm$ 4.520*	102.2 $\pm$ 4.505**
FD-CLCH 50 mg/kg	141.9 $\pm$ 3.500ns	137.0 $\pm$ 2.138*	106.9 $\pm$ 3.877*
FD-CFPE 10 mg/kg	109.0 $\pm$ 5.189***	125.3 $\pm$ 1.546**	108.4 $\pm$ 3.549*
FD-CFPE 20 mg/kg	109.7 $\pm$ 6.307***	137.8 $\pm$ 4.166**	109.0 $\pm$ 3.589*
FD-CFPE 50 mg/kg	117.2 $\pm$ 5.068***	131.1 $\pm$ 3.127**	101.4 $\pm$ 4.702**

All the data were expressed as mean $\pm$ 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. arborescens*=*Nyctanthes arborescens*; *C. gigantea*=*Calotropis gigantea*; FD-NFCH=Fructose-diet-*N. arborescens* flowers chloroform; FD-NLCH=Fructose-diet-*N. arborescens* 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.

Prevention of high-fructose diet induced Insulin resistance by *Nyctanthes arbortristis* and *Calotropis gigantea* in rats**Table 2: Effect of *N. arbortristis* and *C. gigantea* on insulin, FIRI in HFDI insulin resistance in rats.**

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

All the data were expressed as mean $\pm$ 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, ^1H and ^{13}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*.

REFERENCES

- Bray G.A, Nielsen S.J, Popkin B.M. Consumption of high fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am. J. Clin. Nutr.* **79**: 537–543 (2004).
- Davide F, Kaori M., Jean-Marc S., Raj P. Park S. Effect of fructose over-feeding and fish oil administration on hepatic De Nova Lipogenesis and Insulin sensitivity in healthy men. *Diabetes* **54**: 1907–1912 (2005).
- Mehdi H., Mohesen A., Manoochehr M.. Effect of Vanadly Sulfate on Fructose induced Insulin Resistance Rats. *Iranian Biomedicals Journal* **7**(4): 179–182 (2003).
- Bezerra R.M.N., Ueno M., Silva M.S, Tavares D.Q., Carvalho C.R.O., Saad M.J.A, Gontijo J.A.R. A high-fructose diet induced Insulin resistance but not blood pressure change in normotensive rats. *Braz. J. Med. Bio Res.* **34**(9): 115–1160 (2001).
- Sharon S.E. Nancy L.K., Judith S.S. Karen T. Peter J.H.. Fructose, Weight gain, and the insulin resistance syndrome. *Am. J. Clin. Nutr.* **76**: 911–922 (2002).
- Shalam Md., Harish M.S., Farhana S.A.. Prevention of dexamethasone and fructose-induced insulin resistance in rats by SH-01D, a herbal preparation. *Indian J. Pharmacol.* **38**(6): 419–422 (2006).
- Maryam E., Akram E., Hamidreza Z.. Effect of *Salvia officinalis* L. Leaves on serum glucose and insulin in healthy and streptozotocin-induced diabetic rats. *J. Ethnopharmacol.* **(100)**: 310–313 (2005).
- Chetty M., Sivaji K., Rao K.T., *Flowering plants of Chittoor district Andhra Pradesh*, (1st edition, Published by student offset printer, Tirupati, 2008) pp. 193.
- Saxena R.S., Gupta B., Saxena K.K., Srivastava V.K., Prasad D.N.. Analgesic, antipyretic and ulcerogenic activities of *Nyctanthes arbortristis* leaf extract. *J. Ethnopharmacol.* **19**: 193–200 (1987).
- Mathuram V., Kundu A.B., Occurrence of two new ester of 6 β -Hydroxyloganin in *Nyctanthes arbortristis*. *J. Indian Chem. Soc.* **68**: 581–584 (1991).
- Saxena R.S., Gupta B., Lata S.. Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbortristis* leaf extract. *J. Ethnopharmacol.* **81**: 321–325 (2001).
- Talal T.S., Dwivedi S.K., Sharma S.R.. In vitro and In vivo Antitrypanosomal, potential of *Nyctanthes arbortristis* leaves. *Pharmaceutical Biol.* **38**(5): 326–329 (2000).
- Omkar A., Jeeju T.H., Chhaya G.. Evaluation of anti-inflammatory activity of *Nyctanthes arbortristis* and *Onosma echinoides*. *Phcog Mag.* **2**(8): 258–260 (2006).
- Amarite O., Bhuskat P., Patel N. and Gadgoli C.. Evaluation of antioxidant activity of carotenoid from *Nyctanthes arbortristis*. *Int. J. Pharmacol. Biol. Sci.* **1**(2): 57–59 (2007).
- Das S., Sasmal D., Basu S.P. Anti-inflammatory and antinociceptive activity of arbortristoside-A. *J. Ethnopharmacol.* **116**(1): 198–203 (2008).
- Rathore B., Paul B., Chaudhury B.P., Saxena A.K., Sahu A.P., Gutpa Y.K.. Comparative studies of different organs of *Nyctanthes arbortristis* in

- modulation of cytokines in murine model of arthritis. *Biomed. Environ. Sci.* **20**(2): 154–159 (2007).
17. Gupta P., Bajpai S.K., Chandra K., Singh K.L., Tandon J.S. Antiviral profile of *Nyctanthes arborescens* against encephalitis causing viruses. *Indian J. Exp. Biol.* **43**(12): 1156–1160 (2005).
18. Paul B.N., Prakash A., Kumar S., Yadav A.K., Mani U., Saxena A.K., Sahu A.P., Lal K., Dutta K.K.. Silica induced early fibrogenic reaction in lung of mice ameliorated by *Nyctanthes arborescens* extract. *Biomed. Environ. Sci.* **15**(3): 215–222 (2002).
19. Tandon J.S., Srivastava V., Guru P.Y.. Iridoids: a new class of leishmanicidal agents from *Nyctanthes arborescens*. *J. Nat. Prod.* **54**(4): 1102–1104 (1991).
20. Shobha Rani S., Govind K., Bharhavan B.B. Chandan S., Man Mohan S.. Original research article pregnancy interceptive activity of the roots of *Calotropis gigantea* Linn. In rats. *Contraception* **75**: 318–322 (2007).
21. Chitme H.R., Kaushik Ramesh Chandra S.. Studies on anti-diarrheal activity of *Calotropis gigantea* R. BR in experimental animals. *J. Pharm. Pharmacol. Sci.* **7**(1): 70–75 (2004).
22. Chitme H.R., Kaushik Ramesh Chandra S.. Evaluation of analgesic activities of *Calotropis gigantea* extract in vivo. *Asia Pacific J. Pharmacol.* **16**: 35–40 (2005).
23. Chitme H.R., Kaushik Ramesh Chandra S.. Evaluation of antipyretic activity of *Calotropis gigantea* (Asclepiadaceae) in experimental animals. *Phytotherapy Research* **19**(5): 454–456. (2005).
24. Ameeta A. Anupam K.P. CNS activity of *Calotropis gigantea* roots. *J. Ethnopharmacol.* **106**: 142–145 (2006).
25. Nadkarni K.M., Nadkarni A.K. *Indian materia medica*, vol 1. In: Nadkarni K.M. (7th Popular Bombay Prakashan Pvt. Ltd.1976) p.p 237–41
26. Pathak A.K., Argal A.. Analgesic activity of *Calotropis gigantea* flower. Short report. *Fitoterapia* **78**: 40–42 (2007).
27. Rajesh R., Raghavendra Gowda C.D., Nataraju A., Dhananjaya B.L., Kemparaju K., Vishwanath B.S.. Procoagulant activity of *Calotropis gigantea* latex associated with fibrinolytic activity. *Toxicol* **46**: 84–92 (2005).
28. Thakur S., Das P., Itho T., Imai K., Matsumoto T.. Latex extractable of *Calotropis gigantea*. *Phytochem.* **23**: 2085–2085 (1984)
29. Sucharita S., Niranjana P. Sahu S. Mahato B.. Flavonol glycosides from *Calotropis gigantea*. *Phytochem.* **31**(8): 2919–2921 (1992).
30. Sengupta A., Bhattacharya D., Pal G., and Sinha N. K. Comparative studies on calotropins DI and DII From the latex of *Calotropis gigantea*. *Arch. Biochem. Biophys.* **232**(1): 17–25 (1964).
31. Heilmann U. Pai G.P. Hilgenfeld R. and Saenger W.. Crystal and Molecular Structure of the Sulfhydryl Protease Calotropin DI at 3.2Å± a resolution. *J. Mol. Bio.* **161**: 591–606. (1982).
32. Babu V., Ganadevi T., Subramaniam A.. Antidiabetic activity of ethanol extract of *Cassia Klenii* leaf in streptozotocin-induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extract. *Indian J. Pharmacol.* **35**: 290–296 (2003).
33. Dongzhe S. Simon H. Catherine C.Y.P.. Chronic N-acetylcysteine prevents fructose-induced resistance and hypertension in rats. *Eur. J. Pharmacol.* **508**: 205–210 (2005).