

Free Radical Scavenging Activity of *Nyctanthes arbortristis* in Streptozotocin-Induced Diabetic Rats

Nanu Rathod^{1*}, Raghuvver I², Chitme H. R³ and Ramesh Chandra⁴

¹Dept. 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, Azaiba Muscat Sultanate of Oman

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

* Author for Correspondence: nanurathod@rediffmail.com

Abstract

Nyctanthes arbortristis is reported to have a wide range of biological activities such as antidiabetic, antipyretic, anthelmintic, antibilious, expectorant, laxative and is used for treatment of arthritis, obstinate, sciatica, malaria, intestinal worms and also as tonic. The qualitative test of the crude extract shown the presence of alkaloids and flavonoids. The study was aimed to find out the protective effect of *Nyctanthes arbortristis* on lipid peroxidation (LPO) and activity of both enzymatic and non-enzymatic antioxidants in streptozotocin (STZ) induced diabetic rats. The oxidative stress was measured in liver homogenate LPO, Superoxide dismutase (SOD) and Catalase (CAT) levels; blood serum levels of SGPT, SGOT, Alkaline phosphatase (Alk Phos) and cholesterol, triglyceride levels. The significant elevation in LPO, SGPT, SGOT, Alk Phos and cholesterol, triglyceride levels and decreased enzymatic activity of SOD, CAT were the salient features observed in diabetic control rats. Administration of *Nyctanthes arbortristis* leaves and flower chloroform extracts (50, 100 and 200 mg/kg) orally for 27 days caused a significant reduction in LPO, SGPT, SGOT, Alk Phos, cholesterol and triglyceride levels on extracts treated STZ diabetic rats, compared to diabetic control rats. Further more *Nyctanthes arbortristis* extract treated diabetic rats showed significant increase in SOD and CAT enzymatic antioxidant activity when compared to diabetic control rats. The administration of the extracts and glibenclamide (10 mg/kg) improved the activity of both enzymatic and non-enzymatic antioxidants and lipid profile in STZ-induced diabetic rats.

Keywords: Diabetes, *Nyctanthes arbortristis*, lipid profile, Oxidative stress.

INTRODUCTION

Oxidative stress plays an important role in chronic complication of diabetes and is postulated to be associated with increased lipid peroxidation¹. The streptozotocin (STZ) is frequently used to induce diabetes mellitus in experimental animals through its toxic effect on pancreatic β -cells. The cytotoxic action of STZ is associated with the generation of reactive oxygen species causing oxidative damage. Diabetes manifested by experimental animal's model exhibits high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defense systems. Increased oxidative stress and change in antioxidant capacity, observed in both clinical and experimental diabetes mellitus, are thought to be the

etiology of diabetic complication². The disturbances of antioxidant defense systems in diabetes were shown: alteration in antioxidant enzyme such as Superoxide dismutase (SOD) and Catalase (CAT) Glutathione peroxidase (GPx) Glutathione reductase (GR) and impaired glutathione (GSH) metabolism. Anti-oxidants provide protection to living organism from damage caused by uncontrolled production of ROS concomitant lipid peroxidation, protein damage and DNA strand breaking. Ethnomedical literature contains a large number of plants including, *Nyctanthes arbortristis* that can be used against diabetes, Insulin resistance^{3,4} in which reactive oxygen species and free radicals play a major role. Many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes, which may reduce the risk of chronic diseases in diabetic humans⁵. Recently, there has

been an increasing interest in the use of medicinal plants. The plant kingdom has become a target for the search of drugs and biological active lead compounds. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes⁶. Hence, the present study was under-taken to explore free radical scavenging activity of *Nyctanthes arbortristis* on STZ-induced diabetic rats. In recent years, considerable focus has been given to an intensive search for novel type of antioxidants from numerous plant materials⁷. The Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand from patients to use the natural products with antidiabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects⁸.

Nyctanthes arbortristis Linn. (Division: Magnoliophyta; Class: Magnoliopsida; Order: Lamiales; Family: *Oleaceae*), commonly known as Harsingar or Night jasmine, is a well documented plant. It is a native of India, distributed wild in sub-Himalayan region and also found in Indian garden as ornamental plant. The indigenous people of Chittoor district Andhra Pradesh (India) widely use the whole plant for treatment of cancer, root for fever, sciatica, anorexia; bark as expectorant, Leaf for control fever, diabetes and as cholagogue, diaphoretic and anthelmintic. The decoction is used to treat arthritis, malaria, intestinal worms, tonic and laxative⁹⁻¹¹. Antitrypanosomal¹², anti-inflammatory and antioxidant activity has also been exhibited by the various extracts of the plant¹³⁻¹⁵. The *Nyctanthes arbortristis* were tested against Encephalomyocarditis virus (EMCV) and Semliki forest virus (SFV)¹⁶

Previously isolated constituents:

Earlier workers have reported the isolation of polysaccharides, iridoid glycosides, phenylpropanoid glycoside, β -sitosterol, β -amyrin, hentri-acontane, benzoic acid, glycosides, nyctanthoside-a iridoid, nyctanthic acid, Friedelin and lupeol and oleanolic acid and 6 β -hydroxylonganin and iridoid glucosides-arborsides A, B and C, alkaloids, Phlobatanins, terpenoids and cardiac glycosides¹⁷. Iridoid glucosides (arbortristosides-A [1], B [2], C [3], and 6 β -hydroxyloganin [4] isolated from the traditional plants

Nyctanthes arbortristis show Antileishmanial activity in both has been exhibited *in vitro* and *in vivo* test systems¹⁸.

MATERIALS AND METHODS

Drugs and chemicals:

Streptozotocin was obtained from Sigma chemical Co (St Louis, USA). Petroleum ether (40-60 °C) benzene, chloroform, ethyl acetate and methanol (Nice Pvt Ltd, India) and all other chemical are obtained from Sigma and HiMedia laboratories Pvt Ltd.

Preparation of plant:

Nyctanthes arbortristis leaves and flower were collected from widely growing plants from the region of North Karnataka in the months of Sept-October 2005. The plants were identified and confirmed by the Taxonomist of the Basaveshwar Science College, Bagalkot, Karnataka.

The plant material was dried in shade and uniformly powdered by passing through the sieve #. 44 and extracted with petroleum ether (40-60 °C) to defat the preparation, followed by benzene, chloroform, ethyl acetate and methanol by solvent extraction 24 h/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 agent¹⁹. The petroleum ether extraction is known only to defat the plant preparation therefore, the chloroform extract of leaves and flower were selected for the present study. Henceforth, the leaves and flowers extract of *Nyctanthes arbortristis* refers to chloroform extracts of *Nyctanthes arbortristis* leaves and flower

Experimental animals:

Animals

Wistar albino rats (150-200 g) of either sex were used in this study. After one week acclimatizing to laboratory used for investigation. The animals were housed under standard environmental condition of temperature (21 \pm 2 °C), humidity (51 \pm 10%) and a 12 h light-dark cycles with standard pellet diet (Amrut Lab) and water *ad libitum*. All the experiments animals were carried out as per the guidelines of Institutional Animals Ethics Committee (RGE No. 821/a/CPCSEA) of College, after approval (HSK/IAEC.Clear/2004-2005) dated 27/12/04.

Induction of diabetics in rats:

The streptozotocin freshly prepared was dissolved in

citrate buffer (pH 4.5) and rats were made diabetic by injection of a single dose (55 mg/kg) intraperitoneally. They were given 5% of glucose in drinking water for the first 24 h to encounter any initial hypoglycemia. On the third day the animals were checked for serum blood glucose levels, those with higher than 300 mg/dl were used for the experiments¹⁹⁻²⁰.

In our study, a total 54 rats (48 diabetic surviving rats, 6 normal rats) were used. These rats were randomly divided into 9 groups of six rats, after induction of STZ diabetes. Group No.1 (diabetic control) received distilled water in 5% (Tween-80). Group No. 2 received glibenclamide (positive control) at an oral dose (10 mg/kg). Group No. 3 (normal) received distilled water in 5% (Tween-80). Group No. 4, 5 and 6 received chloroform extract of *Nyctanthes arbortristis* leaves (50, 100 and 200 mg/kg). Group No. 7, 8 and 9 received chloroform extract of *Nyctanthes arbortristis* flower oral dose (50, 100 and 200 mg/kg) respectively. The treatment was continued orally daily for 27 days.

Preparation of tissue homogenate:

The tissues were weighed and 10% tissue homogenate was prepared with 0.1 M phosphate buffer (pH. 7.0). After centrifugation at 1000 rpm for 15 m. The supernatant was used to measure protein, thiobarbituric acid reactive substance (TBARS), SOD and CAT.

Analytical procedure:

The blood samples were collected by retro-orbital plexus under anesthesia and used for estimation of blood serum; SGPT, SGOT, Alk Phos, cholesterol and triglyceride were estimated by using commercial diagnostic kit. (Tecodiagnosics USA) on star-21plus semi-autoanalyser.

Protein:

The protein content of the liver homogenate was estimated by the following method²¹. 2.25 ml of 90% alcohol was added to 1 ml of liver homogenate, centrifuged for 3000 rpm for 10 m. The supernatant was discarded and precipitate that settled down was dissolved in 1 ml of 0.1N NaOH, alkaline mixture was added, left for 10 m, 0.5 ml of folin reagent (Phenol reagent) was added and further left for 10 m for colour development, and the absorbance was measured at 610 nm. The protein levels were calculated using standard Bovine serum solution, 200 mg in 100 ml of distilled water.

Lipid peroxidation:

The LPO of the liver homogenate was estimated by the following method²². The reaction mixtures 1 ml liver homogenate, 100 µl of 8.1 % Sodium dodecyl sulfate (SDS) and 600 µl acetic acid solution was left for 2 m at room temperature. Then added 600 µl TBA solution was added. The above solution was boiled for 60 m in water at 95 °C, then cooled with ice cooled water at 4 °C. The mixtures of *n*-butanol and pyridine (15:1, v/v) were added, and the mixture was shaken vigorously and centrifuged at 10,000 rpm for 5 m. The absorbance of the organic layer (upper layer) was measured at 523 nm. The enzyme activity was expressed as units per mg of protein.

Catalase:

The CAT was estimated by the following method²³. The reaction mixture test tube contained 1 ml of Phosphate buffer 0.01 M (pH 7.0) and 0.1 ml of 10% liver homogenate. The reaction was started by addition of 0.4 ml of 2M H₂O₂. The tube was incubated at 37 °C for 10 m. The reaction was stopped by the addition of 2.0 ml of 5% of dichromatic-acetic acids reagent (5% potassium dichromate and glacial acetic acids were mixed in 1:3 ratio). The control was carried out without addition of H₂O₂. The absorbance was read at 620 nm. The CAT activity was expressed as µM H₂O₂ consumed/min/mg of protein. The enzyme activity was expressed as units/mg protein.

Superoxide dismutase:

The SOD was estimated by the following method²⁴⁻²⁵. The assay based on the reduction of Nitro blue tetrazolium (NBT) to water insoluble blue formazan. The liver homogenate 0.5 ml was taken, and 1 ml of 50 mM sodium carbonate and 0.4 ml of 24 µM NBT and 0.2 ml of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 ml of 1 mM hydroxylamine hydrochloride. Absorbance was measured at 560 nm at zero time followed by second measurement after 5 m at 25 °C. The control was simultaneously run without liver homogenates. Units of SOD activity were expressed as the amount of enzymes required to inhibit the reduction of NBT by 50%. The specific activity was expressed in terms of units per mg of protein.

RESULTS

The Table No.1 & 2 depicts the effect of rats with STZ and

treatment of chloroform extracts of *Nyctanthes arbortristis* leaves and flower (50, 100 and 200 mg/kg) on the levels of LPO, SOD, CAT activity in liver homogenates. The treatment of rats with a single dose of STZ at (55 mg/kg) of body weight significantly increased ($P<0.001$) in liver LPO, and blood serum SGPT, SGOT, Alk Phos, and cholesterol, triglyceride in diabetic control rats, on the other hand, SOD, CAT decreased significantly ($P<0.001$) as compared to normal groups of rats due to STZ treatment.

However, treatment of the rats with the chloroform extracts of *Nyctanthes arbortristis* leaves and flower (50, 100 and 200 mg/kg) significantly ($P<0.05-0.001$) reduced lipid peroxidation, SGPT, SGOT, Alk Phos, cholesterol and triglyceride, (except in leaves extract 50 mg/kg in Alk Phos) of which are comparable with the positive control valued. The treatment of the rats with *Nyctanthes arbortristis* extract (50, 100 and 200 mg/kg) respectively, preserves significantly ($P<0.05-0.001$) CAT and SOD activity, which are comparable with glibenclamide was used as positive control.

DISCUSSION

To establish a scientific basis for the utility of this plant in the treatment of diabetes, it was decided to evaluate free radical scavenging activity in STZ-induced diabetic rats. This demonstrates the protection provided by feeding of chloroform extracts of *Nyctanthes arbortristis* leaves and flower (50, 100 and 200 mg/kg) to rats by maintaining the levels LPO, SOD and CAT, biomarker enzymes, cholesterol and triglycerides in STZ-induced diabetic rats. The lipid peroxidation values have been shown to be restored showing antilipid peroxidation effects of the components of chloroform extracts of *Nyctanthes arbortristis* leaves and flower (50, 100 and 200 mg/kg) and glibenclamide (10 mg/kg). Oxidative stress in diabetes is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals²⁶. The SOD and CAT are the two major scavenging enzymes that remove radicals *In vivo*. A decreased activity of these antioxidants can lead to an excess availability of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals (OH), resulting in initiation and propagation of LPO. The SOD can catalyze dismutation

of O_2^- into H_2O_2 , which is then deactivated to H_2O by catalase or SOD works in parallel with selenium-dependent glutathione peroxidase, which plays an important role in the reduction of H_2O_2 in the presence of reduced glutathione forming oxidized glutathione, and it protects cell protein and cell membranes against oxidative stress²⁶.

In this study the SOD and CAT activity significantly decreased ($P<0.001$) in diabetic control group of rats, which may be due to inactivation caused by radicals. However, administration of *Nyctanthes arbortristis* leaves and flower chloroform extracts (50, 100 and 200 mg/kg) and glibenclamide (10 mg/kg) could reverse progress of the disease. The above observations may clearly suggest that increased levels of SOD and CAT of *Nyctanthes arbortristis* extracts has free radical scavenging activity, which may exert a beneficial effect against pathological alterations caused by reactive oxygen species.

The elevation of biomarker enzymes such as SGOT, SGPT, and Alk Phos was observed in diabetic control rats and indicates the hepatic damage²⁷. The hepatic damage was restored hepatocytes and the elevated transaminase was significantly reduced by *Nyctanthes arbortristis* extract. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity²⁸. From this point of view *Nyctanthes arbortristis* leaves and flower chloroform extracts (50, 100 and 200 mg/kg) may act as a hepatoprotective agent²⁹. Diabetic rats showed an important lipolytic activity, due to the insulinopenic state which contributes to maintain the abnormally elevated serum cholesterol and triglyceride levels on STZ diabetic rats³⁰.

The results demonstrated that the *Nyctanthes arbortristis* leaves and flower chloroform extracts (50, 100 and 200 mg/kg) exhibited a potent hypocholesterolemic effect; the possible underlying mechanism is not elucidated at this stage of the study. The previous studies have reported that administration of *Momordica charantia* lead to decrease in cholesterol levels probably by two mechanisms a) by decreasing absorption of cholesterol from intestine by binding with bile acids within intestine and increasing the extraction of faecales bile acids b) by

biosynthesis of cholesterol especially by decreasing the activity of 3-hydroxyl-3-methyl-glutaryl coenzymes A reductase (HMG CoA reductase) an enzyme of cholesterol biosynthesis³⁰. Same mechanism may be appropriate to explain the observed cholesterol and triglycerides lowering activity by *Nyctanthes arbortristis* extracts. Its anti-leishmanial activity has been attributed to its constituent iridoid glucoside, arbortristosides A, B and C and 6β-hydroxyloganin. Given some of the medicinal properties of the plant might be attributed to its

free radical scavenging ability of *Nyctanthes arbortristis* extracts.

CONCLUSION

It was concluded from this study that *Nyctanthes arbortristis* extracts has free radical scavenging activity and improved antioxidant effect was observed. The precise mechanism(s) and site(s) of action as well as constituents of *Nyctanthes arbortristis* will be further determined, including their toxicological effects.

Table 1: Effect of *Nyctanthes arbortristis* leaves and flowers on LPO, SOD, CAT, levels in STZ-induced diabetic rats liver

Treatment	TBARS (nmol/ mg protein)	SOD (Unit/ mg protein)	Catalase (ng/ mg protein)
Control	19.73 ± 2.05***	9.81 ± 0.24***	149.4 ± 14.54***
Gliben 10 mg/kg	9.12 ± 2.23**	14.52 ± 0.70***	314.5 ± 24.10***
Normal	7.48 ± 1.04	18.42 ± 1.59	321.1 ± 22.56
NLCH 50 mg/kg	12.71 ± 0.95*	12.88 ± 0.7140**	281.6 ± 30.95**
NLCH 100 mg/kg	12.85 ± 1.33*	13.81 ± 1.09**	284.4 ± 34.62**
NLCH 200 mg/kg	12.19 ± 1.00**	13.35 ± 0.68***	266.6 ± 24.11**
NFCH 50 mg/kg	12.01 ± 1.07**	13.51 ± 0.61***	254.5 ± 22.55**
NFCH 100 mg/kg	12.07 ± 1.20**	14.85 ± 0.79***	286.2 ± 33.60**
NFCH 200 mg/kg	11.85 ± 1.29**	14.17 ± 0.43***	295.2 ± 27.41***

Control: STZ (Tween-80); Gliben: Glibenclamide; NLCH: *Nyctanthes arbortristis* leaves chloroform; NFCH: *Nyctanthes arbortristis* flower chloroform extract.

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 2: Effect of *Nyctanthes arbortristis* leaves and flower on serum SGPT, SGOT, Alk Phos, Cholesterol, Triglyceride levels in STZ-induced diabetic rats

Treatment	SGPT (U/dl)	SGOT (U/dl)	Alk Phos (U/dl)	Cholesterol (U/dl)	Triglyceride (U/dl)
Control	256.8 ± 21.29***	318.0 ± 26.7***	238.5 ± 9.00***	114.5 ± 4.25**	168.0 ± 9.43**
Gliben 10 mg/kg	68.57 ± 6.52***	119.8 ± 6.14***	186.9 ± 10.85**	94.71 ± 2.91**	122.2 ± 9.27**
Normal	60.9 ± 2.32	64.68 ± 4.67	127.4 ± 2.28	84.36 ± 3.86	115.1 ± 6.82
NLCH 50 mg/kg	81.67 ± 6.31***	154.5 ± 13.91***	226.8 ± 14.89ns	98.52 ± 2.99*	130.6 ± 4.41**
NLCH 100 mg/kg	65.87 ± 3.10***	172.5 ± 13.21***	181.6 ± 8.99**	94.47 ± 4.39**	131.3 ± 1.94**
NLCH 200 mg/kg	68.29 ± 4.34***	184.5 ± 18.26**	184.1 ± 8.81**	97.59 ± 1.17**	117.7 ± 5.94**
NFCH 50 mg/kg	75.96 ± 6.83***	163.8 ± 10.47***	155.8 ± 13.38***	97.89 ± 4.29*	123.9 ± 4.37**
NFCH 100 mg/kg	65.76 ± 4.44***	184.8 ± 13.70**	176.2 ± 10.77**	98.56 ± 2.81*	124.2 ± 7.92**
NFCH 200 mg/kg	57.42 ± 3.62***	191.1 ± 16.01**	184.9 ± 11.17**	91.34 ± 4.42**	131.7 ± 2.36**

CHO: Cholesterol; TRI: Triglyceride; Control: STZ (Tween-80); Gliben: Glibenclamide; NLCH: *Nyctanthes arbortristis* leaves chloroform; NFCH: *Nyctanthes arbortristis* flower 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.

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