

Protective Effect of *Nyctanthes arbor-tristis* Linn. Leaf Extract Against Alloxan-Induced Oxidative Stress in Diabetic Rats

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Abstract

Nyctanthes arbor-tristis Linn. Harsinghar (Nyctanthaceae) is a medicinal plant, which is used traditionally in Indian system of medicine for the treatment of fever, liver disease, rheumatism, respiratory disease, etc. In the present investigation the antidiabetic and antioxidant activity of 50% ethanolic leaf extract of *N. arbor-tristis* (NAEt) were evaluated in diabetic rats induced with alloxan. In comparison to normal control animals, diabetes was related with a decrease in the serum antioxidant enzyme activities such as SOD, CAT and GPx. The diabetic rats were treated with NAEt (100 and 200 mg/kg bw) orally for 21 days. The glucose level in the serum was decreased in the NAEt group in comparison of diabetic control group. In addition, the extract enhanced the activities of SOD ($p < 0.05$), CAT ($p < 0.05 - 0.01$), and GPx ($p < 0.01$); a higher effect at the higher dose. Lipid peroxidation activity, as determined by hepatic thiobarbituric acid-reactive substances (TBARS) was also significantly reduced following NAEt treatment. In summary, the 50% ethanolic extract of *N. arbor-tristis* is found to have promising antidiabetic and antioxidant activity in alloxan induced diabetic rats, possibly by boosting the endogenous antioxidant mechanisms and minimizing the oxidative stress caused by diabetes.

Keywords: *Nyctanthes arbor-tristis*, Alloxan, Diabetes mellitus, Oxidative stress, SOD, Catalase, Glutathione peroxidase.

Introduction

The growing incidence of diabetes mellitus and the diverse complications that are a result of chronic hyperglycaemia have been a driving force towards the identification of more safe and effective plant-based therapeutic options [1]. The accessibility, traditional use and the claimed pharmacological effects of herbal medicines have led to their great attention. The World Health Organization has noted the potential use of traditional and herbal medicines, and recommended their integration in health care [2]. Natural products remain a source of biologically active compounds and are a source of useful leads for pharmaceutical research and drug discovery.

There are various medicinal plants described in the traditional Indian healthcare systems, especially Ayurveda, which are used for treating disorders of high blood glucose. Based on ethnobotanical studies, there are about 800 species of plants that are known to have antidiabetic properties [3]. *Nyctanthes arbor-tristis* Linn. commonly known as Harsinghar is a medicinal plant and is widely distributed in India and also reported from areas like Thailand, Indonesia etc. The leaves have traditionally been used for treating fever, rheumatism, liver disease and respiratory conditions. Other experiments have also reported the plant's pharmacological effects such as anti-inflammatory and anthelmintic activities [4].

In diabetes, the high blood glucose levels may lead to increased formation of reactive oxygen species (ROS) resulting in oxidative stress and disrupt the normal functioning of endogenous antioxidants. This may lead to damage of cells and tissues and is considered a key role contributor in the onset and progression of diabetes associated complications. Thus, the regulation of antioxidant defense mechanisms could be an important aspect of protection from diabetes-induced oxidative damage. Based on this connection between oxidative stress and diabetes, the present research was designed to assess the impact of ethanolic leaf extract (50%) of *Nyctanthus arbor-tristis* on serum antioxidant enzymes activities in serum of diabetes induced rats.

Materials and Methods

Experimental Animals

Albino wistar rats (150–200 g) irrespective of sex, were selected for the experimental work. The animals were housed in polypropylene cages under standardized laboratory environmental conditions, maintaining a temperature of $26 \pm 2^\circ\text{C}$ and relative humidity 45-50%. Standard laboratory diet and drinking water were made available ad libitum throughout the experimental period. Animal experimentation was carried out with the applicable ethical standards and institutional guidelines governing research involving conscious laboratory animals and experimental pain.

Induction and Establishment of Diabetes

Experimental diabetes was induced through injection of alloxan monohydrate at a dose of 150 miligram per kilogram of body mass) intraperitoneally in sterile normal saline (0.9%). In order to reduce the possible risk of an acute hypoglycemia after the injection of alloxan, the animals were offered a 5 per cent glucose solution for the next 24 h [6]. Diabetic diagnosis was performed after twenty one days by measuring the levels of fasting glucose in blood. Blood glucose was measured with Morepen digital glucometer.

Acute toxicity studies (OECD Guideline 423)

An acute oral toxicity assessment was carried out to assess the short-term toxic effects of the test preparation given by the oral route to healthy young adult rodents held under fasting conditions. The test material was gaved a single dose of the material at fixed dosage levels and the animals were observed for up to 15 days for any signs of toxicity and/or mortality. Bodyweight changes were monitored throughout the observation period and at its completion, and then necropsied. In the present study two doses of 100 and 200 miligram per kilogram of body weight were tested, further the animals were closely monitored for dose dependent toxic symptoms and mortality [7].

Hypoglycemic activity

The experimental rats were allocated into five groups and the following treatments were given:

Group I (Normal control): The vehicle (1% carboxymethyl cellulose (CMC)) was given to non-diabetic rats.

Group II (Diabetic control): Diabetic rats were treated with vehicle solution.

Group III (NAEt 100 mg/kg): Diabetes induced rats were orally administered the 50% ethanolic extract of *Nyctanthes arbor-tristis* (NAEt) at a dose of 100 mg/kg b/w.

Group IV (NAEt 200 mg/kg): Diabetic rats received *Nyctanthes arbor-tristis* ethanolic extract (NAEt) orally at a dose of 200 mg/kg b/w.

Group V (Glibenclamide): Diabetic rats received glibenclamide at a dose of 600 $\mu\text{g/kg}$ b/w serving as the standard antidiabetic treatment.

This test was conducted to determine the acute oral toxicity of a test substance after oral administration of predetermined doses to healthy young adult rodents on a fasted state. It was given as a single bolus gavage dose and the rats were observed for up to 15 days for signs of toxicity and death. Body-weight changes were measured throughout the observation period and at the completion of the study, the rats were subjected to necropsy. Fixed doses of 100 and 200 mg/kg b/w weight were used in this research and rats were observed for any dose related toxicity or mortality [7].

Results

Assessment of NAEt on Serum Glucose Levels

The blood glucose content of diabetic rats was significantly increased after alloxan treatment in comparison of the normal control group. Diabetic control group had maintained this hyperglycemic state during the 21-day experimental period [8]. A significant reduction in blood glucose level was monitored with oral ingesti of 100 and 200 miligram per kilogram of 50% ethanolic leaf extract of *Nyctanthes arbor-tristis* (NAEt). The decrease was more marked in the animals fed the 200 200 miligram per kilogram dose, suggesting a dose response effect.

One-way ANOVA revealed a statistically significant difference in serum glucose levels on Days 0 and 21 ($p <$

0.0001) as shown in Table 1. The experimental diabetic model was also responsive and valid, since administration of the standard drug glibenclamide (600 µg/kg) resulted in significant reduction in serum glucose (Table 2 and in Figure 1 [9].

Table 1: One-Way ANOVA Analysis of Serum Glucose Levels in Experimental Groups

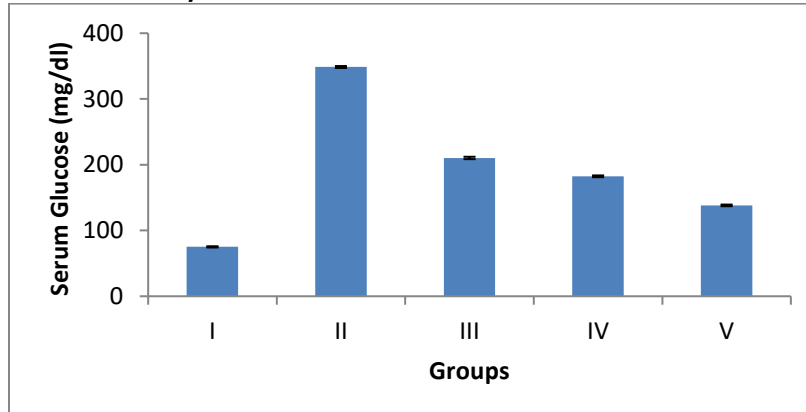
Parameter	Day 0	Day 21
F value	6890	3125
df	4	4
p value	< 0.0001	< 0.0001

Table 2: Assessment of Nyctanthes arbor-tristis Ethanolic Extract (NAEt) on Serum Glucose Levels in Alloxan-treated Rats

Group	Treatment Description	Dose	Serum Glucose (mg/dl) Day 0	Serum Glucose (mg/dl) Day 21
I	Normal control (Vehicle)	10 ml/kg	74.20 ± 0.85	75.10 ± 0.92
II	Diabetic control (Alloxan)	Vehicle	365.40 ± 1.25	348.60 ± 1.48
III	Alloxan + NAEt	100 mg/kg	358.90 ± 1.10	210.30 ± 1.80 ^b
IV	Alloxan + NAEt	200 mg/kg	350.75 ± 0.95	182.40 ± 1.35 ^b
V	Alloxan + Glibenclamide	600 µg/kg	342.60 ± 1.45	138.20 ± 1.25 ^c

*Values are expressed as Mean ± SEM (n =3). aP<0.05, bP<0.01, cP<0.001 compared with diabetic control."

Figure 1: Assessment of Nyctanthes arbortristis Ethanolic Extract (NAEt) on Serum Glucose Levels in Diabetic Rats after 21 Days of Treatment



Assessment of Antioxidant Enzyme Response to NAEt Treatment

In diabetic rats, a marked decrease in enzymatic antioxidant activity was noted, as manifested by lower levels of SOD, CAT and GPx than in normal rats [10]. The activities of all the three antioxidant enzymes (Table 3, Figures 3-5) were significantly improved when treated with Nyctanthes arbortristis Ethanolic Extract (NAEt) 100 and 200 mg/kg. This was dose dependent, with the 200 mg/kg group showing enzyme activity results similar to those of the normal group. There was also an apparent increase in the level of antioxidant enzymes in the glibenclamide-treated group [11].

Assessment of NAEt on Lipid Peroxidation

Significant elevation was observed in hepatic lipid peroxidation in diabetic group as that of normal group. NAEt administration produced a significant reduction in hepatic TBARS concentration, reflecting a notable attenuation of lipid peroxidation. The effect was particularly pronounced in group IV, with the observed response closely

approximating that of the glibenclamide-treated group (Table 3, Figure 2) [12].

Table 3: Assessment of Nyctanthes arbortristis Ethanolic Extract (NAEt) on Hepatic LPO, SOD, CAT and GPx levels in Diabetic Rats (21 Days)

Group	Treatment Description	Dose	LPO (nmol/mg protein)	SOD (units/mg protein)	CAT (units/mg protein)	GPx (mmol/g)
I	Normal control (Vehicle)	10 ml/kg	0.29 ± 0.005	146.20 ± 0.50	45.10 ± 0.85	7.10 ± 0.10
II	Diabetic control (Alloxan)	Vehicle	0.85 ± 0.010	105.60 ± 1.70	16.80 ± 1.05	1.85 ± 0.08
III	Alloxan + NAEt	100 mg/kg	0.60 ± 0.015 ^a	122.40 ± 0.75 ^b	25.90 ± 1.10 ^b	2.90 ± 0.09 ^b
IV	Alloxan + NAEt	200 mg/kg	0.45 ± 0.012 ^a	134.90 ± 0.60 ^b	34.80 ± 0.90 ^a	4.45 ± 0.10 ^a
V	Alloxan + Glibenclamide	600 µg/kg	0.375 ± 0.006 ^c	142.50 ± 0.80 ^c	40.80 ± 0.95 ^c	5.95 ± 0.24 ^c

*Data are presented as Mean ± SEM (n = 3). Statistical significance was considered at ^aP < 0.05, ^bP < 0.01, ^cP < 0.001 in comparison with diabetic control group

Figure 2: Assesment of ethanolic (50%) Nyctanthes arbortristis leaf extract (NAEt) on lipid peroxidation Following 21 days of treatment

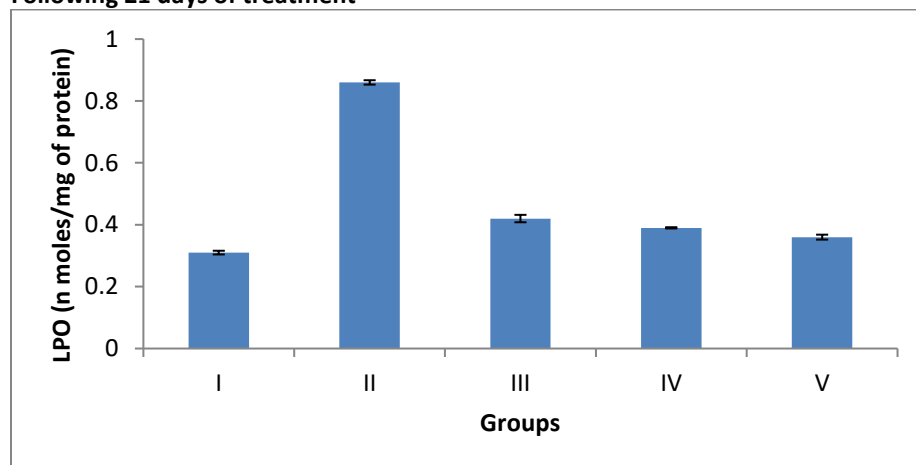


Figure 3: Assesment of ethanolic (50%) Nyctanthes arbortristis leaf extract (NAEt) on superoxide dismutase (SOD) Following 21 days of treatment

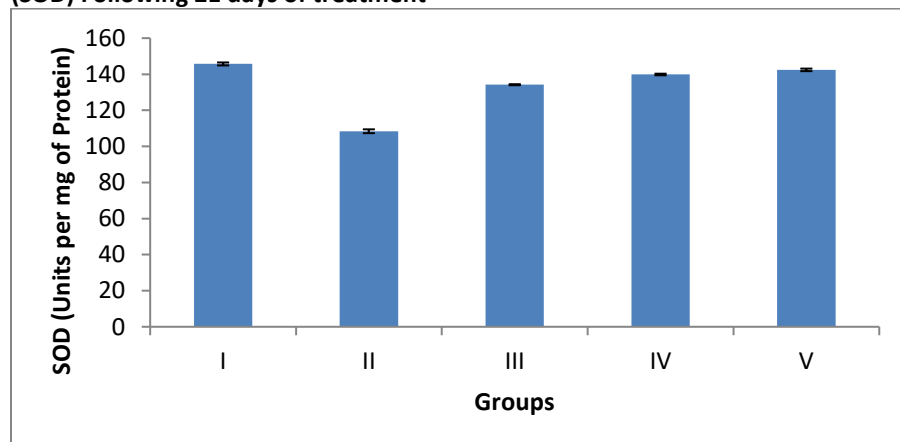


Figure 4: Assesment of ethanolic (50%) *Nyctanthes arbortristis* leaf extract (NAEt) on catalase (CAT) Following 21 days of treatment

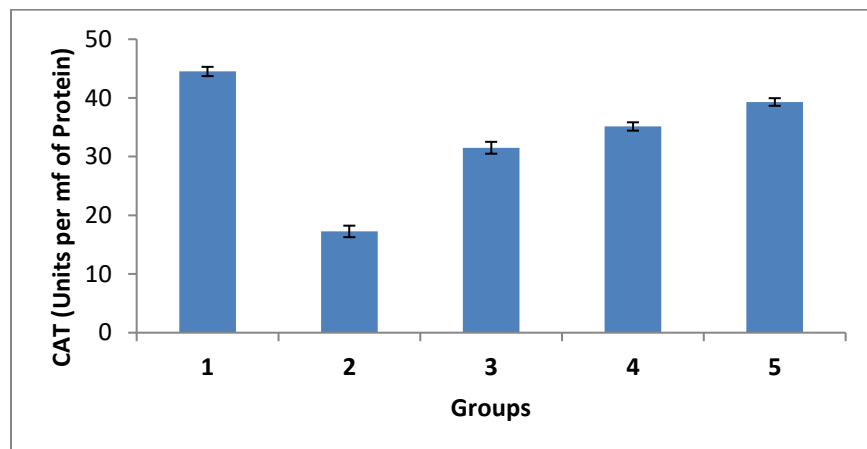
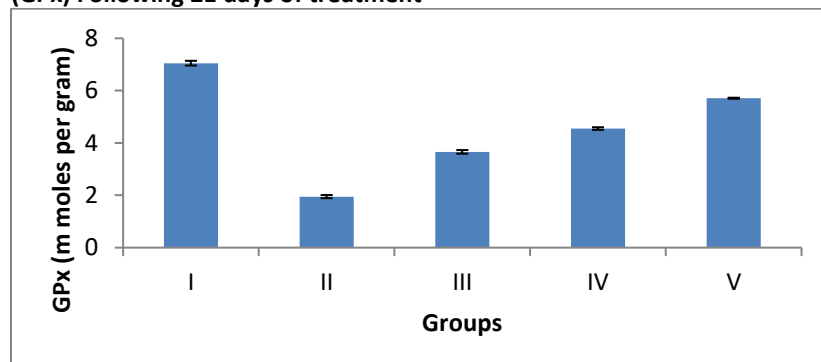


Figure 5: Assesment of ethanolic (50%) *Nyctanthes arbortristis* leaf extract (NAEt) on glutathione peroxidase (GPx) Following 21 days of treatment



Discussion

Diabetes mellitus is chronic metabolic disease which involves hyperglycemia and insulin dysfunction as well as carbohydrate, lipid and protein metabolism. The alloxan-induced diabetes (AIDM) model has been extensively employed in experimental diabetes research to explore the potential of antidiabetic intervention due to the selective pancreatic β -cell damage of alloxan, which leads to reduction in the provision of insulin and chronic hyperglycemia [13].

Alloxan administration resulted in a marked elevation of glucose concentration among the diabetic groups relative to the normal control animals in the present study, thus establishing the diabetic condition. After 21 days oral treatment, there was a remarkable reduction in serum glucose concentration after administration of *Nyctanthes arbor-tristis* ethanolic extract (NAEt). The antihyperglycemic effect observed may be partly due to maintenance or enhancement of the remaining pancreatic β -cell function and/or to increased peripheral glucose utilization and/or to reduced hepatic glucose production [14]. Such mechanisms were not directly explored in the current study, and are thus proposed as potential mechanisms and not demonstrated ones.

The phytochemicals of various groups like flavonoids, phenolic compounds and glycosides have been linked to antidiabetic activity by a number of biological mechanisms such as enhancing insulin sensitivity and impeding carbohydrate digestion [15]. Therefore, the glucose lowering activity observed after NAEt administration might be due to the presence of bioactive constituents in *N. arbor-tristis*.

Diabetes is linked to oxidative imbalance, and its progression. Chronic hyperglycemia can lead to the overproduction of reactive oxygen species (ROS) that could harm the cells and reduce the body's own antioxidant capacity [16]. To support this relation, the diabetic control animals of the present study also found to have reduced activities of SOD, CAT and GPx, indicating the impairment of antioxidant defense system under diabetic conditions.

The SOD enzyme is a role player in cellular antioxidant defense mechanism in that it transforms the superoxide radicals into hydrogen peroxide. This generates hydrogen peroxide, which then is neutralised by enzymes like CAT and GPx, thus reducing the oxidative damage to cellular structures [17]. The antioxidant enzyme activities were restored after NAEt treatment indicating that the extract could possibly help to reduce the damage caused by the imbalance of antioxidants in experimental diabetes.

The administration of NAEt significantly increased the activities of the endogenous antioxidant enzymes (SOD, CAT and GPx) suggesting that the extract can positively modulate the endogenous antioxidant defence system and thus reduce the excessive antioxidant burden due to diabetes.

Oxidative stress also brings about lipid peroxidation that may damage cell membranes and cause tissue damage. In the present study, higher concentration of TBARS in the liver was observed in diabetic control animals related to increased lipid peroxidation. NAEt significantly reduced TBARS levels indicating that the extract could afford protection to hepatic tissue against oxidative damage to the membranes. In experimental diabetes, similar antioxidant and lipid protective activities have been reported for formulations made from medicinal plant extracts [18].

The dual anti-hyperglycemic and anti-oxidative effects after NAEt treatment are of particular pharmacological relevance. Relaxed hyperglycemia may facilitate the production of ROS, thereby adding to oxidative injury that plays a role in the development of diabetic complications such as nephropathy, neuropathy and cardiovascular abnormalities [19]. The observed decrease in the oxidative stress markers hence implies that the *Nyctanthes arbor-tristis* extract may have additional benefits apart from glucose regulation, by preventing injury to cells and tissues during diabetes.

Hence, the present findings show that NAEt is a potential antidiabetic and antioxidant agent in alloxan induced diabetic rats. However, additional research is needed to determine which phytoconstituents are responsible for these activities and to elucidate the cellular and molecular mechanisms that account for the pharmacological effects of the extract.

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