

EFFECT OF *NYCTANTHES ARBORTRISTIS* LINN. LEAVES AGAINST STREPTOZOTOCIN INDUCED OXIDATIVE STRESS IN RATS

ALTAF HUSAIN¹, UJJWAL TIWARI², VIJAY SHARMA³, AMIT KUMAR⁴ NADEEM RAIS²

¹Department of Pharmacology, Azad Institute of Pharmacy and Research, Lucknow.

²Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University, Moradabad

³PRIST University, Tamil Nadu

⁴Faculty of Pharmacy, Sagar Institute of Technology and Management, Barabanki.

Abstract

Nyctanthes arbortristis Linn. (Fam: Nyctanthaceae) is commonly known as 'Harsinghar' and its leaves are traditionally used to treat fevers, rheumatism, liver disorders and as expectorant. The present work is aimed to evaluate the effect of leaves of *N. arbortristis* on antioxidant enzymes status in streptozotocin induced diabetic rats. STZ diabetic rats showed decreased levels of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in serum as compared to normal. Diabetic animals were treated with 50% ethanolic extract of *N. arbortristis* Leaves (NAEt; 100 and 200 mg/kg b.w) for three weeks. Oral administration of NAEt showed the marked reduction in elevated level of serum glucose as compared with diabetic group. NAEt significantly increased SOD ($p < 0.05$), CAT ($p < 0.05$, $p < 0.01$), GPx ($p < 0.01$) in serum at a dose of 100 and 200 mg/kg. Further, NAEt significantly inhibited the lipid peroxidation by reducing TBARS in liver. The results suggested that oral administration of NAEt possesses significant antidiabetic and antioxidant potential. It was concluded that antidiabetic effect of *N. arbortristis* may due to its antioxidant compounds which neutralizes the oxidative stress in diabetic condition.

Keywords: *Nyctanthes arbortristis*, Streptozotocin, superoxide dismutase, catalase, serum glucose

Introduction

The current interest in and demand for herbs is a worldwide phenomenon, WHO currently encourages, recommends and promotes traditional/ herbal remedies in national healthcare programmes because such drugs are easily available at low cost, are comparatively safe and people have faith in such remedies[1]. From research point of view, natural products are rapidly being utilized as source for drug discovery and development[2,3]. The use of herbs in the management of diabetes mellitus has been prevalent in Indian society from a long time. The ethnobotanical information reports about 800 plants that may possess anti-diabetic potential[4]. Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques[5,6,7,8,9]. Several medicinal plants such as *Galega officinalis*[10] have reported to possess potential hypoglycemic activity in Indian system of medicines. There have been several reviews on the hypoglycemic medical plants[11,12], more particularly use of Indian botanicals for hypoglycemic activity[13,14,15]. *Nyctanthes arbortristis* Linn. (Fam: Nyctanthaceae) is commonly known as 'Harsinghar' and native to India, Thailand and Indonesia, It is cultivated in gardens almost through out India and in many tropical countries. Traditionally used to treat fevers, rheumatism, liver disorders and as expectorant. It also possesses anti-inflammatory and anthelmintic activity[16,17].

Objective:

The objective of the present work is aimed to evaluate the effect of 50% ethanolic extract of *N. arbortristis* leaves on antioxidant enzymes status in streptozotocin induced diabetic rats.

Material and Method:

1. Animals

Sprague-Dawley rats (150-185g) and Swiss albino mice (20-25 gm) of either sex and of approximately the same age were procured from the animal house of Central Drug Research Institute, Lucknow. They were kept in the departmental animal house at $26 \pm 2^\circ\text{C}$ and relative

humidity 44 – 56 % in polypropylene cages. All experiments were performed in the morning according to current guidelines for investigation of experimental pain in conscious animals[18].

2. Induction of Diabetes[19]:

A freshly prepared solution of STZ (50 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) was injected intraperitoneally which increased the blood glucose level more than 200mg/dl in albino rats.

3. Acute toxicity studies (OECD Guideline 423)

This test involves the administration of a simple bolus dose of test substances to faster healthy young adult rodents by oral gavage, observation for upto 15days after dosing and recording of body weight and the necropsy of all the animals. In this method pre-specified fixed doses of the test substances were used ie, 5mg/Kg, 50mg/Kg, 300mg/Kg, 2000mg/Kg and the mortality due to these doses were observed.

4. Experimental Design: The five groups of rats were used in this experiment as follows

- Group I - Control rats received vehicle solution (1% CMC)
- Group II - Diabetic control rats received vehicle solution
- Group III - Diabetic rats treated with extract 100 mg/kg b.wt
- Group IV - Diabetic rats treated with extract 200 mg/kg b.wt
- Group V - Diabetic rats treated with Glibenclamide 600 µg/kg b.wt

Diabetic animals were treated with NAEt 100 and 200 mg/kg b.w for three weeks. After treatment, rats were sacrificed, blood serum was separated. SOD, CAT, GPx were estimated in serum and LPO was estimated in liver.

Result:

STZ diabetic rats showed decreased level of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in serum as compared to normal. Oral administration of NAEt showed the marked reduction in elevated level of serum glucose as compared with diabetic group (Table.1, Fig.1). NAEt significantly increased SOD ($p < 0.05$), CAT ($p < 0.05$, $p < 0.01$), GPx ($p < 0.01$) in serum at a dose of 100 and 200 mg/kg. Further, NAEt significantly inhibited the lipid peroxidation by reducing TBARS in liver (Table.2, Fig.2). Effect of 50 % aqueous alcoholic extract of plants on serum glucose, lipid

*Corresponding Author:

Altaf Husain

Azad Institute of Pharmacy and Research, Lucknow

Ph: 09889704101

E-mail: altafhamdard@gmail.com

profile and antioxidant status in alloxan induced diabetic rats was studied. Based on this, potentiation of dreaded disease like diabetes mellitus may shows a ray for better protocol for future treatment. The efficacy of 50% ethanolic extract of leaves of *Nyctanthes arbortristis* in experiment showed the significant decrease in the blood glucose level, increase the antioxidant efficacy in alloxan induced diabetes.

The present study showed that the 50% ethanolic extract of leaves of *Nyctanthes arbortristis* possess strong anti-hyperglycemic and antioxidant effect in STZ induced diabetic condition.

Conclusion:

In recent year several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies confirmed the efficacy of several medicinal plants in the modulation of oxidative stress associated with diabetes mellitus. The results suggested that oral administration of NAET possesses significant antidiabetic and antioxidant potential. It was concluded that antidiabetic effect of *N. arbortristis* may be due to its antioxidant compounds which neutralizes the oxidative stress in diabetic condition.

Table. 1 Anti-diabetic effect (Glucose level) of 50% ethanolic extract of leaves of *Nyctanthes arbortristis* (NAEt) on STZ induced diabetic rats after 0 and 21 days.

Groups	Treatment	Dose	0 day (mg/dl)	After 21 days (mg/dl)
I	Control normal	10 ml/kg	72.33 ± 0.71	72.66 ± 0.88
II	Diabetic control	50 mg/kg	392.66 ± 1.14	374.75 ± 1.43
III	STZ+ NAET 100	100 mg/kg	380.16 ± 0.94	200.5 ± 1.73 b
IV	STZ+ NAET 200	200 mg/kg	372.33 ± 0.66	189.83 ± 1.22
V	Glibenclamide	600 µg/kg	352.00 ± 1.59	142.16 ± 1.35
One-way ANOVA				
F			7,411	2,739
df			4	4
p			<0.0001	<0.0001

Value are expressed as Mean ± SEM of 6 rats in each group and 4 rats in Diabetic control group.

b = P < 0.01 compared with diabetic control group.

Fig. 1 Anti-diabetic effect (Glucose level) of 50% ethanolic extract of leaves of *Nyctanthes arbortristis* (NAEt) on STZ induced diabetic rats after 21 days

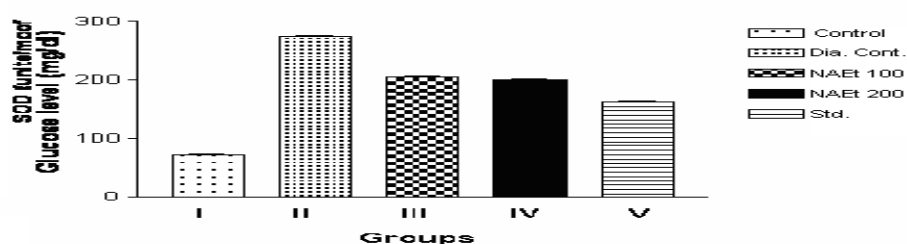


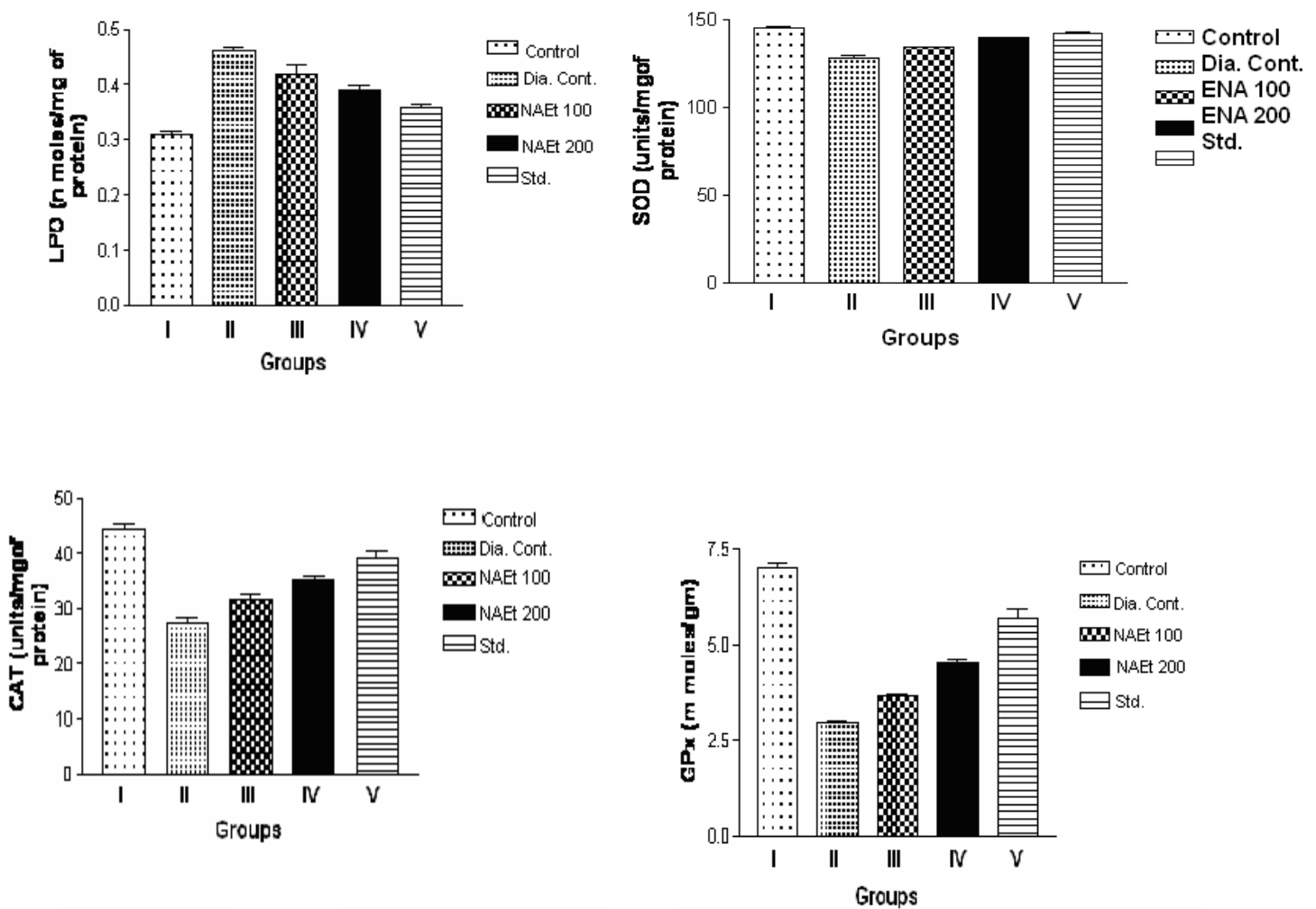
Table. 2 Effect of 50% ethanolic extract of leaves of *Nyctanthes arbortristis* (NAEt) on lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase in 21 days

Groups	Treatment	Dose	LPO (n moles/mg of protein)	SOD (units/mg of protein)	CAT (units/mg of protein)	GPx (m moles/gm)
I	Control normal	10 ml/kg	0.31 ± 0.006	145.70 ± 0.42	44.51 ± 0.81	7.05 ± 0.09
II	Diabetic control	50 mg/kg	0.86 ± 0.008	108.39 ± 1.65	17.26 ± 1.10	1.95 ± 0.07
III	STZ+ NAET 100	100 mg/kg	0.42 ± 0.014a	134.18 ± 0.66	31.51 ± 1.12 b	3.66 ± 0.07 b
IV	STZ + NAET 200	200 mg/kg	0.39± 0.009a	139.85 ± 0.51	35.14 ± 0.83 a	4.55 ± 0.08
V	Glibenclamide	600 µg/kg	0.36 ± 0.004	142.40 ± 0.88 a	39.31 ± 0.94 b	5.71 ± 0.25
One-way ANOVA						
F			36.42	65.27	44.97	129.9
df			4	4	4	4
p			< 0.0001	<0.0001	< 0.0001	<0.0001

Values are expressed as Mean ± SEM of 6 rats in each group and 4 rats in diabetic control group.

a= P < 0.05, b = P < 0.01 compared with diabetic control group.

Fig. 2 Effect of 50% ethanolic extract of leaves of *Nyctanthes arbor-tristis* (NAEt) on lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase in 21 days



Acknowledgment

The first author is thankful to NATIONAL BOTANICAL RESEARCH INSTITUTE, Lucknow.

References:

- Handa, S.S., Herbal raw materials and traditional remedies. Eastern Pharmacist, 38:23-25,1995
- Buss, A.D. and Waigh, R.D., Natural products as leads for new pharmaceuticals. In Berger's medicinal chemistry and drug discovery, 5th edi., 1: 983-1033,1995
- Zhong Shu, Y., Recent natural products based drug development: A pharmaceutical industry perspective. Journal of natural products, 61:1053-1071,1998
- Alarcon-Aguilar, F.J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar- Contreras, A., Contreras-Weber, C.C., Flores-Saenz, J.L., Study of the anti-hyperglycemic effect of plants used as antidiabetics. Journal of Ethnopharmacology 61 (2): 101- 110, 1998.
- Saifi, A.Q., Shinde, S., Kavishwar, W.K., Gupta, S.R., Some aspects of phytochemistry and hypoglycemic actions of Pterocarpus marsupium. Journal of Research in Indian Medicine 6 (2): 205- 207, 1971.
- Mukherjee, K., Ghosh, N.C., Datta, T., Coccinia indica Linn. As potential hypoglycemic agent. Indian Journal of Experimental Biology 10 (5): 347- 349, 1972.
- Coimbra, T.C., Danni, F.F., Blotta, R.M., da Periar, C.A., Guedes, M.D., Graf, R.G., Plants employed in the treatment of diabetes mellitus; results of an ethnopharmacological survey in Porto Alegre, Brazil. Fitoterapia, 63 (4): 320- 322, 1992.
- Ajit kar, Choudhary, B.K., Bandhopadhyay, N.G., Preliminary studies on the inorganic constituents of some indigenous hypoglycemic herbs on oral glucose tolerance test. Journal of Ethnopharmacology, 64: 179-184y, 1999.
- Jafri, M.A., Aslam, M., Javed, K., Singh, S., Effect of Punica granatum Linn. (flowers) on blood glucose level in normal and alloxan-induced diabetic rats. Journal of Ethnopharmacology, 70 (3): 309-314, 2000.
- Rang, H.P., Dale, M.M., The Endocrine System Pharmacology, Second ed.. Longman Group Ltd, UK, 504- 508, 1991.
- Ivorra, M.D., Pay'aa, M., Villarb, A., A review of natural products and plants as potential hypoglycemic drugs. Journal of Ethnopharmacology, 27: 243-275, 1989.
- Rahman, A.U., Zaman, K., Medicinal plants with hypoglycemic activity. Journal of Ethnopharmacology, 26: 1-55, 1989.
- Grover, J.K., Yadav, S., Vats, V., Medicinal plants of India with hypoglycemic potentials. Journal of Ethnopharmacology, 81: 81-100, 2002a.

14. Saxena, A., Vikram, N.K., 2004. Role of selected Indian plants in management of type 2 diabetes: a review. *Journal of Alternative and Complementary Medicine* 10, 369–378.
15. Mukherjee , P.K., Maiti ,K., Mukherjee ,K., Houghton, P.J., Leads from Indian medicinal plants with hypoglycemic potentials, *Journal of Ethnopharmacology*, 106 :1–28, 2006.
16. Parotta, J.A., *Healing plants of peninsular India*, CAB International, Wallingford, U.K., 548-549, 2001.
17. Nair,C.K.N., Mohanan, N., *Medicinal plant of India*, NAG Publication,Delhi, India,316-317, 2001.
18. Zimmerman. M., Ethical guidelines for investigations of experimental pain in conscious animals, *Pain*, 16: 109-110, 1983.
19. Vijayakumar, M., Govindrajan, R., Rao, G. M. M., Rao, Ch. V., Shirwaikar, A., Mehrotra, S., Puspagandan, P., Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress, *Journal of Ethnopharmacology* 104 (2006) 356-361.