ORIGINAL ARTICLE

Anti-hyperglycemic and antioxidant activities of the ayurvedic drug *Nisha kathakathadhi* churnam in alloxan-induced hyperglycemic rats

C. H. Jithendra, P. Muralidharan[1]
Department of Pharmacology, Bapatla College of Pharmacy, Bapatla, Guntur, Andhra Pradesh - 522 101, [1]Department of Pharmacology, C.L. Baid Metha College of Pharmacy, Chennai, Tamil Nadu - 600 096, India

The anti-hyperglycemic and antioxidant potential of *Nisha Kathakathadhi* Churnam (NKC), an ayurvedic formulation, was estimated in alloxan-induced, hyperglycemic rats. Various enzymatic antioxidants, such as, superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione and lipid peroxidation were estimated in the heart and pancreas of hyperglycemic rats. The effect of NKC on blood glucose levels was estimated on days 0, 10, 20 and 30 of a 30-day study. A marked decrease in the blood glucose levels ($P < 0.001$) was observed in hyperglycemic rats and the decreased activities of the key antioxidant enzymes increased to near normal ($P<0.01$) levels and the increased lipid peroxidation decreased in diabetic rats upon NKC treatment. These results suggest that NKC has promising antidiabetic and antioxidant activities in alloxan-induced diabetes.

Key words: Alloxan, antioxidants, diabetes, *Nisha kathakathadhi* churnam

INTRODUCTION

Diabetes mellitus (DM) is one of the major health problems in the world today. The incidence of diabetes is affecting people from all walks of life. As per the recent estimate, there are approximately 142 million diabetics all over the world and the number is expected to double in the next 20 years.

Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycemia. Several distinct types of DM exist and are caused by a complex interaction of genetics, environmental factors and life-style choices. The metabolic disregulation associated with DM causes secondary pathophysiological changes in multiple organ systems, which are associated with oxidative stress and damage to tissues. Excessive generation of free radicals on unsaturated fatty acids has been implicated in the pathogenesis of vascular diseases and the normal antioxidant defence mechanism is insufficient in the regulation of this increased oxidative stress. Hence antioxidants from other sources are to be provided to counteract the oxidative stress.

In Ayurveda, one of the oldest traditional medicinal systems, diabetes is referred to as 'Madhumeha' and many formulations are prescribed for the treatment of the same. Some of the formulations have been studied for their efficacy, while many others are yet to be screened. *Nisha kathakathadhi* Churnam (NKC) is one of the ayurvedic polyherbal drugs widely used in the southern parts of India for the management of diabetes. In addition, this drug was said to be effective in the treatment of diabetes-related complications.[1] As there are no scientific reports on the same, it was taken up for scientific validation in Alloxan-induced hyperglycemic rats.

Nisha kathakathadhi Churnam, consists of the following plants *Strychnos potatorum, Aerva lanata, Ixora coccinea, Symplocos cochinensis, Salacia reticulata* and *Vetiveria zizanioides* in a definite proportion. The formulation is suspended in warm water (1 : 4 ratio) and administered to the animals by the oral route.

MATERIALS AND METHODS

Animals

Male wistar rats (150-200 g) obtained from the animal house of the C.L. Baid Metha College of Pharmacy, Chennai, were maintained at a constant temperature of 26 ± 2°C and humidity of 30-40%, with 12 hours light/dark cycle throughout the experimentation period. The animals were fed with pellet feed (supplied by the T.N. University for Veterinary and Animal Sciences, Chennai) and water was provided *ad libitum*. Animals were acclimatized to the laboratory conditions one week prior to the initiation of experiments. The experimental design was approved by the Institutional Animal Ethics Committee and the study was performed according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Address for correspondence: Asst. Prof. C. H. Jithendra, Department of Pharmacology, Bapatla College of Pharmacy, Bapatla, Guntur, Andhra Pradesh - 522 101, India. E-mail: jithu_indra@rediffmail.com

Received: 27-06-2008; **Accepted:** 13-09-2008; **DOI:** 10.4103/0973-8258.49378

guidelines for the use and care of experimental animals.

Effect of NKC on Glucose Levels in Normal Fasting Rats

After 18 hours of fasting, the animals were divided into four groups each containing six rats. Group I animals served as the control, while Group II animals were treated with the standard drug Glibenclamide (1 mg/kg p.o.), NKC was administered orally in doses of 500 mg/ kg and 1000 mg/ kg p.o., respectively, to Group III and Group IV rats. The blood glucose levels were estimated before and 4, 8 and 12 hours after administration of the standard and test drugs (Ghosh, 1984), using GLUCOMEN GLYCO (manufactured by M/s A. Menarini Industries Farmaceutiche Reunite, Italy), a one-touch blood glucose monitoring system. The blood collection was done by the tail-vein method.[2]

Effect of NKC on Alloxan-induced Hyperglycemia

Another set of animals were divided into five groups (n = 6), and to Groups II-V, alloxan (120 mg/kg i.p.) in normal saline was administered to the overnight-fasted rats.[3] After three days of alloxan treatment, the hyperglycemic status of the rats was confirmed by using a one-touch glucometer,[4] before subjecting them to a study. Group III animals were given the standard drug Glibenclamide (0.5 mg/kg/day p.o.) for 30 days,[5] while group III and IV received NKC orally at a dose of 500 and 1000 mg/kg/day,[6] respectively, for 30 days. Group I rats served as normal control and Group II as diabetic control. The blood glucose levels were monitored on days 0, 10, 20 and 30. On day 30 all the animals were sacrificed using a high dose of ether anaesthesia, the heart and pancreas were removed quickly, washed with ice-cold saline and preserved.

Effect of NKC on Antioxidant Enzymes and Lipid Peroxidation

The tissues of the heart and pancreas of the rats were cut into small pieces and homogenized using Tris-buffer (0.01 M, pH 7.4) at 4°C to get 10% homogenate. The levels of antioxidant enzymes superoxide dismutase (SOD),[7] catalase (CAT),[8] glutathione peroxidase (GPx),[9] reduced glutathione (GSH)[10] and lipid peroxidation (LPO)[11] were estimated as per standard procedures.

Statistical Analysis

The data obtained in the studies were subjected to one way analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using the Dunnet's t test. $P < 0.05$ was considered to be significant. All the values were expressed as mean ± SEM.

RESULTS

Effect of Single Administration of NKC on Blood Glucose Levels in Normal Rats

In normal rats, a single administration of NKC (500 mg and 1 g/kg) significantly brought down the blood glucose levels after 4 hours, when compared with control animals. At the eighth hour the reduction was almost comparable with that of the standard drug, Glibenclamide. The results are tabulated in Table 1.

Effect on Blood Glucose Levels and Antioxidants After Multiple Administrations of NKC in Hyperglycemic Rats

The alloxan-induced hyperglycemic condition was maintained in these animals till they were sacrificed on the thirtieth day. In the animals treated with Glibenclamide and NKC (500 mg/kg and 1 g/kg), the reduction in the blood glucose levels were significant on days 10, 20 and 30. At a higher dose (1000 mg) NKC revealed highly significant reduction in the blood glucose level. The results are tabulated in Table 2.

Likewise, in the antioxidant studies too, the rats in the

Table 1: Effect of NKC on normal blood glucose levels in rats

Groups		0 h	4 h	8 h	12 h
I	Control	84.25 ± 6.35^{A}	86.46 ± 11.73^{Bns}	84.94 ± 9.06^{Bns}	85.24 ± 3.
II	Glibenclamide (1 mg/kg)	81.40 ± 5.26^{A}	53.47 ± 3.77^{B**}	45.64 ± 5.62$^{B\#}$	54.42 ± 3.94^{B**}
III	NKC (500 mg/kg)	86.51 ± 3.24^{A}	63.42 ± 5.53^{B*}	55.31 ± 4.12^{B**}	64.75 ± 8.21^{B*}
IV	NKC (1 gm/kg)	82.73 ± 4.46^{A}	58.68 ± 7.95^{B*}	52.44 ± 6.35^{B**}	62.53 ± 6.34^{B*}
F			7.26	10.25	6.42

Values are mean ± SEM of six observations (d.f. = 4, 25); Values of B are compared with values of A; *$P < 0.05$; **$P < 0.01$; $^{\#}P < 0.001$; ns-nonsignificant

Table 2: Effect of NKC on blood glucose levels of hyperglycemic rats

Groups		0 Day	10th Day	20th Day	30th Day
I	Control	73.1 ± 8.2^{A}	74.1 ± 4.5^{Bns}	71± 5.1^{Bns}	77 ± 3.5^{Bns}
II	Diabetic control	231.3 ± 35.7^{A}	243.5 ± 21.5^{Bns}	267.8 ± 33.54^{Bns}	288.3 ± 41.3^{Bns}
III	Glibenclamide (1 mg/kg)	240.3 ± 26.3^{A}	151.8 ± 14.3$^{B\#}$	118.2 ± 7.12$^{B\#}$	105.7 ± 7.3$^{B\#}$
IV	NKC (500 mg/kg)	236.2 ± 25.1^{A}	177 ± 18.6$^{B\#}$	131.2 ± 16.3$^{B\#}$	127.7 ± 14.2$^{B\#}$
V	NKC 1 gm/kg)	241.2 ± 44.5^{A}	156.7 ± 19.8$^{B\#}$	127.3 ± 12.67$^{B\#}$	108.7 ± 8.5$^{B\#}$
F			51.48	77.87	97.44

All values are expressed as mean ± SEM of six observations; Values of B are compared with values of A; $^{\#}P < 0.001$; ns-nonsignificant

disease-control group showed a decrease in the levels of antioxidant enzymes (SOD, CAT, GSH and GPX) and increase in LPO in both pancreas and heart. Treatment with the standard drug and NKC brought down these values to near normal and the activity was highly significant. The results are tabulated in Tables 3 and 4.

DISCUSSION

Alloxan and the product of its reduction, dialuric acid, are reported to generate superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. Thereafter, highly reactive hydrogen radicals are formed by the Fenton reaction. The action of these reactive oxygen species (ROS) with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β-cells.[12]

Apart from the ROS diabetes also increases LPO by initiating the β oxidation of fatty acids of the cell membranes, and is mediated by the fatty acyl coenzyme, A oxidase enzyme,[13] resulting in membrane function impairment and altering membrane permeability. Diabetic retinopathy is characterized by increased capillary permeability in the retina by leakage of fluorescein, and increased permeability in the kidney causes increased urinary loss of albumin, which predicts eventual renal failure.[14]

Increased LPO under diabetic conditions may be due to increased oxidative stress in the cells as a result of the depletion of antioxidant scavenging enzymes like SOD, CAT, GSH and GPx. Superoxide dismutase has been recognized to play an important role in the body defence mechanisms against the deleterious effect of oxygen free radicals in the biological systems.[15] Catalase and (GPx) are the scavenging enzymes that remove the toxic free radicals by detoxifying the H_2O_2 concentration. GSH is involved in the synthesis of important macromolecules and in the protection against ROS.[13] Decreased GSH content contributes to the pathogenesis of complications associated with diabetic states. Since, alloxan exhibits a high affinity for the SH-containing cellular compounds, GSH, Cysteine and protein-bound sulphydryl groups (including SH-containing enzymes) are very susceptible to its action. Apart from this, one more important action of Alloxan on Glucokinase, a SH-containing compound essential for glucose-induced insulin secretion, is the increased vulnerability of the sulphydryl groups of Glucokinase to Alloxan, resulting in decreased GSH levels in diabetic states.[12]

The activities of these enzymes are lowered by the ROS that are released by Alloxan and its metabolite dialuric acid. In the present study there has been a decrease in the levels of antioxidant enzymes SOD, CAT, GSH and GPX, which may be due to the increased ROS that also causes destruction of β cells of pancreas.

Administration of *Nisha kathakathadhi* churnam (NKC) showed a significant increase in the levels of antioxidant enzymes in the heart and pancreas and a decrease in LPO was observed. This antioxidant effect of the extract may be of help in patients with diabetic retinopathy and nephropathy. Hence from our results it is suggested that NKC has a significant protective effect against Alloxan-induced Type I diabetes in rats, and this can be attributed to the combined effect of various chemical constituents of various plants used in NKC. The probable mechanism involved in the treatment of Type I diabetes and its efficacy in the treatment of Type II diabetes are yet to be determined, to provide the

Table 3: Effect of NKC on antioxidants and LPO in rat pancreas

Groups		SOD	CAT	GSH	GPX	LPO
I	Control	$4.1 \pm 0.2^{A\#}$	$12.7 \pm 0.8^{A\#}$	$17.3 \pm 2.4^{A\#}$	$26.9 \pm 4.6^{A\#}$	$31.5 \pm 5.6^{A\#}$
II	Diabetic control	2.1 ± 0.16	5.5 ± 0.4	7.13 ± 1.8	13.6 ± 3.1	53.7 ± 6.3
III	Glibenclamide (1 mg/kg)	$3.7 \pm 0.3^{B\#}$	$11.5 \pm 2.3^{B\#}$	$16.1 \pm 2.05^{B\#}$	$24.2 \pm 6.4^{B**}$	$35.1 \pm 7.8^{B\#}$
IV	NKC (500 mg/kg)	$3.0 \pm 0.6^{B**}$	$8.7 \pm 1.9^{B**}$	$11.8 \pm 2.4^{B*}$	$20.1 \pm 2.6^{B*}$	42.9 ± 8.4^{Bns}
V	NKC (1 gm/kg)	$3.4 \pm 0.2^{B**}$	$11.08 \pm 1.2^{B\#}$	$15.2 \pm 1.1^{B**}$	$22.7 \pm 3.1^{B**}$	$36.4 \pm 4.6^{B\#}$
F		11.4	52.01	29.13	24.4	17.78

All values are expressed as mean ± SEM, n-6; A-Group I vs Group II B-Group II vs Group III, IV and V; *$P < 0.05$; **$P < 0.01$; #$P < 0.001$

Table 4: Effect of NKC on antioxidants and LPO in rat heart

Groups		SOD	CAT	GSH	GPX	LPO
I	Control	$12.8 \pm 1.6^{A\#}$	$6.2 \pm 1.3^{A\#}$	$12.6 \pm 1.5^{A\#}$	$1.08 \pm 0.2^{A\#}$	$0.6 \pm 0.06^{A\#}$
II	Diabetic control	6.2 ± 2.8	2.9 ± 0.4	5.9 ± 0.5	0.6 ± 0.07	1.2 ± 0.11
III	Glibenclamide (1 mg/kg)	$12.1 \pm 3.4^{B\#}$	$5.39 \pm 0.8^{B**}$	$11.1 \pm 1.7^{B\#}$	$0.9 \pm 0.2^{B**}$	$0.7 \pm .03^{B**}$
IV	NKC (500 mg/kg)	$8.7 \pm 1.6^{B**}$	$3.6 \pm 0.3^{B*}$	$7.5 \pm 0.9^{B*}$	$0.7 \pm 0.2^{B*}$	$0.9 \pm 0.09^{B*}$
V	NKC(1 gm/kg)	$11.7 \pm 2.1^{B\#}$	$4.8 \pm 1.6^{B**}$	$10.6 \pm 1.3^{B\#}$	$0.8 \pm 0.08^{B**}$	$0.7 \pm 0.05^{B**}$
F		42.32	5.88	13.10	4.18	3.09

All values are expressed as mean ± S.E.M, n-6; A-Group I vs Group II B-Group II vs Group III, IV and V.; *$P < 0.05$; **$P < 0.01$; #$P < 0.001$; Units: - SOD: Units/mg protein; CAT: μ moles of H_2O_2 utilized/min/mg protein; GPx: μg of NADPH oxidized/min/mg protein; GSH: μg/gm wet tissue; LPO: n moles of MDA liberated/min/mg protein

role of NKC in the treatment of diabetes.

REFERENCES

1. Sharma RK, Das B. Agnivesa's Charaka Samhita (English Translation), Vol. 2, Varanasi: Chowkhamba Sanskrit Series Office; 1996. p. 426-8.
2. Nagarajan NS, Murgesh N, Tirupathy Kumaresan P, Rasha N, Murali A. Anti diabetic and anti hyperlipemic effects of Clemeo feline. Fitoterapia 2005;76:310-5.
3. Koshla P, Bhanwara S, Singh J, Seth S, Srivastava RK. A study of hypoglycemic effect of Azadirachta indica in normal and alloxan diabetic rats. Indian J Physiol Pharmacol 2000;44:69-74.
4. Venkatesh S, Reddy GD, Reddy BM, Ramesh M, Rao AV. Anti hyperglycemic activity of Caralluma attenuate. Fitoterapia 2003;74:274-9.
5. Vijayvargia R, Kumar M, Gupta S. Hypoglycemic effect of aqueous extract of Enicostemma littorale blume (chotta chirayata) on alloxan induced diabetes mellitus in rats. J Exp Biol 2000;38:781-4.
6. Ghosh MN, Fundamentals of experimental pharmacology. Calcutta: Scientific Book Agency; 1984. p. 153-5.
7. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dimutase. J Biol Chem 1972;247:3170-5.
8. Bergmeyer HV, Gowehn K, Grassel M. Methods of enzymatic analysis. New York: Academic Press Inc; 1974. p. 438-41.
9. Ncheles TF, Boles TA, Allen DM. Erythrocyte glutathione - peroxidase deficiency and hemolytic disease of newborn. Pediatrics 1968;42:319-21.
10. Leszczyńska A. Effect of Reduced Glutathione on pharmacokinetics and distribution of Rifamycin in rats. Pharmacol 1981;30:71-6.
11. Okhawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
12. Szkudelski T. The mechanism of Alloxan and Streptozotocin action on β cells of the rat pancreas. Physiol Res 2001;50:536-46.
13. Manonmani G, Bhavapriya V, Kalpana S, Govindasamy S, Apparanatham T. Antioxidant activity of Cassia fistula (Linn.) flowers in Alloxan induced diabetic rats. J Ethnopharmacol 2005;97:39-42.
14. John CP, Williams G. Pathophysiology of Microvascular disease: An overview. Text book of Diabetes, Vol. 2. USA: Black Well Scientific Publications; 2003. p. 526-31.
15. Kakkar P, Das B, Viswanathan PN. A modified spectropotometric assay of superoxide dismutase. Indian J Biochem Biophysics 1984;21:130-2.

Source of Support: Nil, **Conflict of Interest:** None declared.