RESEARCH ARTICLE
Evaluation of the effect of Nishamalaki on cataract in diabetic rats

Pallawi Khatakar, Jayshree Dawane, Madhura Bhosale, Vijaya Pandit, Pradnya Padalkar

Department of Pharmacology, Bharati Vidyapeeth Deemed to be University Medical College, Pune, Maharashtra, India

Correspondence to: Vijaya Pandit, E-mail: drvapandit@gmail.com

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder, leading to many complications. Cataract is important complication causing impairment of vision. Many factors play a role in cataract formation in diabetic individuals. Only treatment for cataract is surgery, having limitations. There is a need to explore for inexpensive, non-surgical approaches for prevention of cataract. The present study was planned to evaluate the effect of Nishamalaki (NA) on diabetic cataract.

Aims and Objectives: This study aims to study the prophylactic and therapeutic effect of NA on cataract and its mechanism of action.

Materials and Methods: Diabetes was induced in 42 Wistar rats with low-dose streptozotocin (STZ) followed by high-fat high-fructose diet. NA treatment initiated in six rats immediately after STZ administration (NA prophylactic Group II). Once diabetes was developed rats divided into six groups and received respective treatment for 8 weeks: Group I – diabetic control, Group III – NA therapeutic, Group IV – glimepiride, Group V – chloramphenicol, Group VI – glutamine, and Group VII – epalrestat. Then, estimation of aldose reductase (AR) and antioxidant enzymes in blood and lens was done.

Results: In Group I, cataract was initiated in the 2nd week and progressed to Stage 3 or 4 by the 8th week. No cataract was developed in NA prophylactic and chloramphenicol group. Delayed initiation and progression were seen in NA therapeutic and epalrestat, compared to glimepiride and glutamine group. NA was seen to work by antihyperglycemic, antioxidant, AR inhibition, and probably by CYP 450 inhibition.

Conclusion: NA is useful in the prevention and treatment of cataract in diabetic animals, working through many mechanisms.

KEY WORDS: Diabetes Mellitus; Cataract Score; Oxidative Stress; Nishamalaki

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder in which blood sugar level remains high over prolonged period. According to the WHO, India has become the country with the fastest growing population of diabetic patients.[1]

Important aspect of diabetes is the occurrence of multisystem effects in the form of diabetic complications over due course of time. Diabetic complications are more common in uncontrolled diabetes. Cataract, which occurs in old age, is preponed in diabetic individuals.[2] Patients with DM are 2–5 times more likely to develop cataract than non-diabetic.

Cataract is a loss of the normal transparency of the crystalline lens. It is one of the leading causes of blindness worldwide[3] and impairs quality of life. There is no treatment for prevention or reduction in the progression of cataract in modern medicine. The only treatment for cataract is surgery, generally providing excellent sight restoration. In developing country like India, resources in terms of personnel and cost are the important limitation. It has been shown that postponement of cataract by 10 years can reduce the need for cataract surgery by almost 50%.[4] Thus, there is a need to explore for inexpensive, non-surgical approaches for prevention/treatment of cataract.
Exact mechanism of the formation of cataract is not known. Some factors which are known to hasten its occurrence are – prolonged hyperglycemia, increase in the oxidative stress, and increased activity of some enzymes such as aldose reductase (AR) and Cytochrome P450 (CYP450).[5]

In Ayurveda, Nishamalaki (NA) treatment is initiated on the diagnosis of DM and is continued lifelong. NA is a combination drug containing Emblica officinalis and Curcuma longa. Individually, these components are shown to have multiple actions in diabetes[6,7] and diabetic complications.[8,9] NA is shown to have antidiabetic action in vitro,[10] preclinical,[11] and clinical study.[12] It is also shown to have antioxidant action. NA is not evaluated for its efficacy in diabetic complications.

Considering the multitude of actions of NA, it was hypothesized that it would be effective in delaying initiation and maturation of diabetic cataract. The present study was undertaken to evaluate this hypothesis.

MATERIALS AND METHODS

The animal experiments were conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals in the Central Animal House of BVDU Medical College, Pune. The Institutional Animal Ethics Committee approval was obtained before initiating the experiment-BVDUMC/1395/2014-2015.

Animals

A total of 42 Wistar rats of 150–200 g of either sex were selected as experimental animals and housed in standard cages and provided with food pellets and water ad libitum.

Induction of Diabetes in Rats[13]

Streptozotocin (STZ) dissolved in citrate buffer (0.01 M, pH 4.5) was given to all rats in the dose of 35 mg/kg as an intraperitoneal injection. It was followed by high-fat (HF), high-fructose diet. HF diet was prepared by soaking pellets in the mixture of coconut oil and vanaspati ghee in the proportion of 2:3 overnight, before use. Fructose (10%) was supplied in drinking water.[14]

Grouping of Animals

In six rats, NA treatment (0.9 gm/kg orally once a day) was started immediately after the injection of STZ. This was NA prophylactic group (Group 2-NA-P).

For remaining animals, development of diabetes was confirmed with the measurement of blood glucose level (BSL) by glucometer which was recalibrated. Animals showing BSL >250 mg/dl were considered diabetic and were randomly assigned to different groups (n = 6).

• 1 – Diabetic control (DM-C)
• 3 – NA therapeutic (NA-T) – 0.9 gm/kg
• 4 – Glimepiride (Glim) – 0.1 mg/kg
• 5 – Chloramphenicol (Chlor) – 86 mg/kg
• 6 – Glutamine (Glut) – 1 gm/kg
• 7 – Epalrestat (Epal) – 4 mg/kg

Doses were extrapolated from human doses.[10] Respective drug treatment was given orally once a day for 8 weeks.

Blood glucose was monitored every 15 days with glucometer and cataract development was observed every 4th day, using ophthalmoscope.

Stages of Cataract[15]

• Stage 0 – Clear lens
• Stage 1 – Faint peripheral opacity
• Stage 2 – Irregular peripheral opacity with slight involvement of the center of the lens
• Stage 3 – Dense nuclear opacity
• Stage 4 – A mature cataract – dense opacity in both cortex and nucleus

Blood Sample Collection

After 8 weeks, blood sample was collected by retro-orbital puncture for the estimation of malondialdehyde (MDA), superoxide dismutase (SOD), and catalase activities.

Extracapsular Lens Extraction and Preparation of Lens Homogenate [Figure 1]

Animals were sacrificed and lenses were removed by extracapsular extraction.[16]

Tissue culture medium-199, powder was reconstituted to 1 L with distilled water. The pH of the medium was maintained at 7.2–7.4. All material and solutions required for lens culture were autoclaved. At the end of 48 h, the lenses were removed from the culture media and gently rolled on filter paper.

Each lens was then homogenized in 0.1 M sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 10,000 × g for 30 min at −40°C in a refrigerated centrifuge. The supernatant was collected and stored at −20°C until further use. The supernatant was used for estimation of AR enzyme, MDA levels, and SOD and catalase activity.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 6. All results were expressed as mean ± standard error of the mean SEM. The data were analyzed by one-way ANOVA followed by Tukey’s multiple comparison test.
RESULTS

Six animals were included in each group, and therefore, a total of 12 eyes per group were evaluated.

Initiation of Cataract

In diabetes control group, cataract development was seen from the 2nd week. Cataract development started in the 6th week in glimepiride group, 7th week in glutamine and epalrestat groups, and 8th week in NA therapeutic group. No cataract developed in NA prophylactic and chloramphenicol groups [Figure 2].

Stages of Cataract at the End of Study (8th week)

In diabetic control group, cataract developed in all the eyes. Eight eyes showed Stage 3 cataract and four eyes showed Stage 4 cataract. In NA prophylactic (NA-p) group, no cataract was developed. No cataract was seen in chloramphenicol group also. In NA therapeutic (NA-T) group, in eight eyes, no cataract was seen and four eyes showed Stage 1 cataract. In glimepiride group, no cataract developed in six eyes, whereas in remaining eyes, Stage 1 cataract was seen. In glutamine group, cataract developed in all the eyes – Stage 1 in 10 eyes and Stage 2 in two eyes. In epalrestat group, eight eyes showed no cataract, Stage 1 and Stage 2 cataract developed in two eyes each [Figure 3].

Blood Sugar Levels at the End of the 8th week

Only NA-P, NA-T, and Glim groups showed significant decrease in BSL, in comparison to DM-C. Significant BSL reduction was not obtained in Chlo, Glut, and Epal groups [Figure 4].

AR Enzyme Activity at the End of 8 Weeks

AR enzyme activity was significantly reduced in all the groups in comparison to DM-C. In comparison to DM-C, NA-p and therapeutic groups showed the highest reduction (P < 0.001) in AR. Effect of Glim, Chlo, and Epal was comparable and significant (P < 0.01) followed by Glut (P < 0.05) [Figure 5].

MDA Activity at the End of 8 Weeks

Three animals in chloramphenicol group died in the 8th week, so lenses from this group were not available for the evaluation of oxidative stress and antioxidant action.

Significant reduction in MDA activity was observed in all the groups in comparison to DM-C group. NA groups showed significantly more reduction in comparison to other groups. Although the difference was not statistically significant, prophylactic NA was more effective than therapeutic. MDA activity in serum and lens was comparable in all the groups [Figure 6].

SOD Activity at the End of 8 Weeks

SOD-antioxidant activity was significantly increased in both NA (P < 0.001) and Glut (P < 0.001) groups compared to DM-C, and in these groups, SOD activity in the lens and serum was comparable. Serum of Glim and Epal groups showed (P < 0.05) increase in SOD, but in the lens, increase was not significant. NA-T showed comparable activity to Glut but was significantly less (P < 0.01) in comparison to NA-P. Glim and Epal groups had comparable SOD activity, which was significantly lower (P < 0.001) than that of NA groups. Due to mortality in the Chlo group, SOD activity in the lens could not be studied [Figure 7].

Catalase Activity at the End of 8 Weeks

Catalase activity was significantly increased (P < 0.001) in both NA groups in comparison to DM-C. Activity in NA-T
was less \((P < 0.01)\) in comparison to NA-P. Catalase activity in other drug-treated groups – Glim, Glut, and Epal – was significantly lower \((P < 0.001)\) in comparison to NA groups. Plasma and lens catalase activity was comparable in all the groups [Figure 8].

**DISCUSSION**

With increasing incidence of diabetes, mainly Type II, and its complications, there is urgent need to have medications in the prevention and treatment of this disease and its complications. Conventionally, we have two types of models – genetic and non-genetic. Genetic models are pathologically more akin to Type II DM. However, they are very expensive and are difficult to maintain. Non-genetic models are cost effective but do not have all the characteristics of human type II diabetes. Rather, these models resemble Type I diabetes more than Type II diabetes.\(^{[17,18]}\)

Type II DM is a heterogeneous disorder characterized by insulin resistance, dyslipidemias, and reduced \(\beta\)-cell function. Recently, there is increasing trend to use models using combination of diet and drug to induce diabetes. HF...
produces central obesity and dyslipidemia; high fructose HF diet leads to insulin resistance; and STZ causes β-cell dysfunction. It is considered that IR is the initial pathological derangement and so HFHF diet followed by small dose of STZ is commonly used to induce Type II diabetes and its complications. However, in the United Kingdom Prospective Diabetes Study (UKPDS), it was seen that by the time of diagnosis, diabetic patients were seen to have lost >50% of β-cell secretory capacity. Hence, in the present study, STZ, HF, and high fructose were started simultaneously.

Effect of curcumin is extensively studied for prevention and treatment of diabetes and its complications. It has shown to be effective in reducing β-cell damage, reduce inflammatory changes in fat and other tissues, and improve insulin resistance. Zhang et al., 2013, have summarized all the actions of curcumin. Antioxidant, hypolipidemic, antidiabetic, and nephroprotective actions have been summarized by Bhandari et al. It has been reported that activity of curcumin is improved if used with Vitamin C (Vit C) and amla rich source of Vit C. Rao et al. reported antidiabetic and antioxidant activity of NA in diabetic rats.

Cataract is one of the early manifestations of diabetic complications. Increased glucose levels in aqueous humor may induce variety of changes in lens, leading to the formation of cataract. Activation of AR enzyme, leading to the accumulation of sorbitol, is considered to be one of the main factors in the occurrence of diabetic cataract. In addition, the formation of advanced glycation end products and oxidative stress are the other mechanisms shown responsible for the development of diabetic cataract.

Initiation and progression of cataract are found to be hastened in patients of DM. In comparison to control, early development of cataract was observed in animal studies also. In the present study, initiation [Figure 2] and progression of cataract [Figure 3] were delayed in both the NA groups, in comparison to diabetes control group.

Studies (Diabetes Complications and Control Trial and UKPDS) have established that hyperglycemia is the initiating cause of the diabetic tissue damage. Most of the cells of our body are able to control the entry of glucose inside the cells. Those cells which fail to do this—such as retina, kidney, and neurons suffer from damage due to hyperglycemia. Changes of cataract formation are more in diabetic patients in comparison to non-diabetic individuals. Glimepiride, a potent antidiabetic agent, was used to evaluate antidiabetic activity of NA and also to compare cataract reducing effect arising with reduction of BSL. Blood sugar was only marginally increased in NA-P group and it never was >250 mg% which is considered as diabetic level in rats and this might be the reason for no development of cataract in this group. STZ specifically acts on β cells of pancreas and produces oxidative damage. Therefore, drugs having antioxidant action would be effective in reducing the damage. NA is reported to exert antioxidant action. In this group, diabetes did not develop probably due to less β-cell damage due to antioxidant action of NA and so no cataract developed in this group.

Glimepiride was more efficacious (P < 0.001) than NA-T (P < 0.01) in reducing BSL [Figure 4], but cataract reduction was more in NA-T group [Figure 1]. Moreover, cataract reduction without reduction in BSL was seen in epalrestat, glutamine, and chloramphenicol groups. This indicated that though reduction of hyperglycemia can reduce the initiation and progression of cataract, other equally important mechanisms exist in controlling diabetic cataract formation.

In diabetes, glucose is flushed by polyol pathway (AR enzyme) to sorbitol and its further degradation is slower. Hence, it gets accumulated in the lens. In addition, sorbitol being water soluble, is not easily removed out of the cell. The accumulated sorbitol increases the osmotic pressure inside the lens, producing collapse and liquefaction of lens proteins. Activation of AR is widely accepted mechanism of diabetic cataract formation. Epalrestat was included in the study to evaluate the effect of NA on AR and also to compare the cataract reducing effect of inhibition of AR. AR inhibition was seen in all the drug-treated groups. Good correlation was found between inhibition of AR and slowing in the initiation and progression of cataract. In comparison to Epal (P < 0.01), NA-P and NA-T (P < 0.001) more significantly inhibited AR and also cataract. Although AR activity is not studied for NA, curcumin and emblica are shown to possess this activity. It is reported that curcumin and emblica reduce AR even in the low (dietary) dose also, which does not reduce blood sugar level. This indicates that curcumin and amla have potent AR inhibitory action. In STZ-induced diabetic rats, turmeric was found to be more effective than curcumin. NA is a combination of turmeric and amla. Efficacy of NA in the present study could be the combined effect of both these agents. Glutamine (P < 0.05) had lesser effect on both AR and on cataract. Chloramphenicol was equieffective on AR but more effective in reducing cataract than epalrestat indicating that it probably has additional anti-cataract action.

Electrons generated during metabolism of glucose through tricarboxylic acid cycle produce voltage across the mitochondrial membrane. In hyperglycemia, more electrons are generated than can be used, leading to the production of superoxide molecules and the development of oxidative stress. Brownlee concluded that hyperglycemia-induced mitochondrial superoxide production activates many damaging intracellular pathways, leading to diabetic complications. Oxidative stress damages almost all biomolecules such as DNA, proteins, and lipids. For demonstration of oxidative stress in diabetic complications, in this case, cataract, a validated biomarker, is required.
MDA is one of the biomarkers for evaluation of oxidative stress in diabetes. Hence, we evaluated MDA activity in the serum and lens. Hyperglycemia-induced overproduction of superoxide is a continuous process and it is observed that catalytic antioxidant, SOD/catalase mimetic agents, reduces this process. Hence, we evaluated SOD and catalase activity in the serum and lens. Glutamine was included in the study to evaluate the effect of NA on oxidative stress and antioxidant action and also to compare the cataract reducing effect due to these actions.

All animals in chloramphenicol group died by the end of the 8th week and so oxidative stress and antioxidant activity could not be evaluated for this group. MDA activity was reduced in all other drug-treated groups in comparison to diabetic control group. NA produced maximal effect followed by glutamine. In comparison to glutamine, MDA was significantly (P < 0.01) less in NA groups, indicating reduced oxidative stress. Similar reduction in oxidative stress is reported for curcumin, amla, and also for NA in diabetes. Increase in SOD was comparable, but increase in catalase was significantly more (P < 0.01) in NA groups in comparison to glutamine. Surya et al. reported increase in SOD with amla but not with curcumin when used alone. Increase in SOD in our study is probably due to the combined action of both the agents. NA appears to have stimulated the expression of antioxidant enzymes—both SOD and catalase—more than glutamine. Antioxidant activity is the sum total of all antioxidant enzymes, which was more in NA group compared to glutamine. Increase in antioxidant activity probably reduced oxidative stress and also initiation and progression of cataract. Antioxidant action in serum and lens was comparable indicating that NA and glutamine penetrate lens effectively.

CYP450 enzymes play major role in oxidation, dealkylation, and epoxide formation. Almost all mammalian tissues contain one of these cytochromes in various organelles. Liver being the organ of metabolism has many types of CYP enzymes in large quantity. CYP enzymes present at other cellular levels are site specific. Nakamura et al. have studied lens CYP such as CYP1A1, CYP2B2, CYP2C11, and CYP2E1. Like liver CYP enzymes, cellular CYP can also be stimulated or inhibited. CYP enzyme status is reported to affect the occurrence and progression of cataract. Chloramphenicol has inhibitory action mainly on CYP2C11 and CYP 1A1. Chloramphenicol was included in the study to evaluate the effect of NA on lens CYP enzymes and also to compare the cataract reducing the effect of CYP inhibition.

AR uses nicotinamide adenine dinucleotide phosphate (NADPH) as cofactor. Electron transfer from NADPH depends on cellular CYP450 system. Action of AR, therefore, is dependent on CYP enzymes. Kavitha et al. showed that diltiazem, CYP inhibitor, delayed occurrence of cataract and an early onset of cataract was observed with pioglitazone which is a CYP inducer. Chloramphenicol, which inhibits lens CYP enzymes, did not allow cataract to develop and was more effective than NA. Chloramphenicol had no antihyperglycemic action [Figure 4], inhibited AR less effectively than NA [Figure 5]. The action on cataract was comparable to NA-P group. However, in NA-P group, no hyperglycemia was present. This indicates that a CYP450-mediated pathway may have more important role, independent of hyperglycemia, in the development of cataract.

The discrepancy between glycemia and diabetic complication incidence and severity is considered to be due to the “metabolic memory.” According to this theory, hyperglycemia-induced mitochondrial oxidative stress leads to variety of intracellular changes such as production of advanced glycation end products and changes in gene expression, leading to self-propagated intracellular vicious cycle maintaining oxidative stress where continued hyperglycemia is not required. This might be the reason for our finding that more than just reducing hyperglycemia; AR inhibition, antioxidant action, and CYP enzyme inhibition were more important. Among these, CYP enzyme inhibition was most effective. Robertson et al. presented the evidence that, in the context of hepatic steatosis, CYPs 2E1 and 4A could generate the “second hit” of cellular injury, particularly when antioxidant reserves are depleted. Lens CYPs might be working as the second hit for cataract formation. Chloramphenicol, which specifically inhibits lens CYP, was highly effective but was lethal and so cannot be considered for long-term treatment.

**CONCLUSION**

NA is effective in reducing development and progression of cataract and acts by multiple mechanisms: reducing hyperglycemia, AR inhibition, and antioxidant action. CYP inhibition per se was not evaluated.

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