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OXIDATIVE ECLIPSE: PROTEIN REDOX IMBALANCE IN DIABETIC CATARACT – AN INVITRO STUDY ON GOAT LENSES

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Abstract
Background: Long-lived proteins (LLPs) are essential for cellular architecture, especially in tissues like the eye lens. However, due to their extended lifespan, they are susceptible to post-translational modifications like oxidative damage and glycation. Maintaining the redox status within cells is crucial for LLP functionality. Cataracts, caused by the clouding of the eye's natural lens, are a primary cause of visual impairment and blindness. Chronic hyperglycemia in diabetes increases the risk of cataract formation due to metabolic disturbances. **Objectives:** This study investigates the redox status of lens proteins and its role in diabetic cataract formation. By evaluating carbonyl and sulfhydryl groups, the study aims to understand the oxidative damage to lens proteins. Oxidative modifications, such as carbonylation and cross-linking, lead to protein aggregation and reduced solubility, resulting in lens opacity. **Methods:** Using in-vitro goat lens models, this research explores oxidative changes and antioxidative response mechanisms under diabetic conditions. Goat lenses, due to their physiological similarities to human lenses, provide valuable insights into diabetic cataractogenesis. A total of 64 goat lenses were divided into experimental diabetic cataract (n=32) and control groups (n=32), incubated in glucose-rich and normal conditions, respectively. Protein carbonyl and sulfhydryl contents were measured using spectrophotometric techniques. **Results:** Oxidation of lens protein was found to be significantly associated with diabetic cataract. In diabetic cataract, a statistically significant increase in the level of protein carbonyl and significant decrease in the level of protein sulfhydryl was seen as compared to controls. **Conclusion:** The findings underscore the importance of maintaining redox balance within the lens to prevent or

delay diabetic cataract formation. Oxidation of lens proteins is a crucial factor in diabetic cataractogenesis, suggesting the potential for antioxidant therapies in cataract prevention.
Key words: Cataract, Protein Redox, Protein Oxidation, Protein Carbonyl, Protein Sulfhydryl

Introduction

Long-lived proteins (LLPs) are integral components of cellular architecture and function, particularly in tissues with low cellular turnover such as the lens of the eye[1]. These proteins, due to their extended lifespan, are susceptible to various post-translational modifications, including oxidative damage and glycation[2]. The redox status within cells, which reflects the balance between oxidants and antioxidants, plays a pivotal role in maintaining the functionality of LLPs. Disruption of this balance can lead to oxidative stress, a condition characterized by excessive reactive oxygen species (ROS) that can damage cellular components, including proteins, lipids, and DNA[3].

Cataracts, characterized by the clouding of the eye's natural lens, are a leading cause of visual impairment and blindness worldwide. Among the various etiologies of cataract formation, diabetes mellitus stands out as a significant risk factor. Diabetic cataract, a common complication of diabetes, results from the metabolic disturbances associated with chronic hyperglycemia.It has recently been proven that cataracts arise 2–5 times more commonly and at a relatively early age in diabetics compared to non-diabetic counterparts[4].

The lens's ability to operate properly is mostly maintained by the protein redox state. In biological systems, proteins are important targets for free radicals because of their abundance and high reaction rate constants. It's likely that systemic diseases like diabetes can cause proteins to oxidise by changing the redox state of proteins. One of the primary changes resulting in cell damage and tissue abnormalities in cataractogenesis has been shown to be an increased build-up of oxidised proteins[5]. It has been suggested that evaluating the carbonyl and sulfhydryl groups of proteins is a useful indicator of the protein redox state in the lens[6]. Since sulfhydryl proteins are well recognised for their structural and functional roles in the lens, oxidation causes a decrease in their abundance, whereas carbonyl proteins indicate a measure of the oxidative damage to those molecules.

Previous studies have highlighted that oxidative modifications to lens proteins, such as carbonylation and cross-linking, play a crucial role in the onset of diabetic cataract[7]. These alterations lead to protein aggregation and reduced solubility, ultimately resulting in lens opacity. Investigating the redox status of lens proteins and its connection to the progression of diabetic cataract is vital for crafting effective therapeutic approaches.

In-vitro models provide valuable insights into the molecular mechanisms underlying diabetic cataractogenesis. Goat lenses are commonly used in such studies due to their anatomical and physiological similarities to human lenses. These models allow for controlled manipulation of experimental conditions, facilitating the investigation of specific pathways involved in cataract formation. By exploring oxidative changes and antioxidative response mechanisms under diabetic conditions, this research aims to uncover the molecular foundation of diabetic cataract formation. The hypothesis is that maintaining redox equilibrium within the lens is essential in preventing or delaying the development of cataracts in diabetic individuals.

Materials & Methods

Study design: Laboratory based experimental study.

Sample Size: In all, 70 fresh goat lenses were used in the experiment. Six lenses out of the total 70 were taken out of the research owing to haziness and damage. Lenses were divided into two groups. Experimental diabetic cataract group (n=32) in which lenses were placed in KRB buffer with 55 mM of glucose and control group (n=32) here lenses were placed in KRB buffer with 5.5 mM of glucose.

Sample Collection: Eyeballs from goats were collected at the slaughterhouse and brought in an ice box to the lab. The intracapsular lens extraction technique was used to remove the lenses from the eyes. Lenses were carefully put on sterilized Petridishes. The lenses were cultured for 72 hours using the "Lens Organ Culture Technique" in KRB buffer pH 7.8 with Cefixime 500 mg.

Lenses from each group were removed after 72 hours of incubation, and a 0.1 M sodium phosphate buffer solution was made to create lens homogenate (pH 7.4). The homogenate was centrifuged at 10,000 x g for 30 min. at -4 °C in a refrigerated centrifuge. Until needed, the supernatant was collected and stored at -20°C.

The modified Levine et al. method was used to measure the carbonyl protein in the lens homogenate[8]. Whereas a modified version of the Ellman technique was used to spectrophotometrically determine the amount of protein sulphydryl (P-SH) in the lens[9].

The present study was approved by the Institute Research Committee and Institute Ethics Committee (IRC/IEC).

Data analysis

For the analysis of data GraphPad Prism (Version 6) was employed. The findings of biochemical parameters were compared between experimental diabetic cataract and normal control using the student's "t" test. All findings were presented as mean and SD. A p value <0.05 was considered statistically significant.

Results

Mean and standard deviation of carbonyl protein content in the lens of normal control and experimental diabetic cataract is depicted in Figure 1. The concentration of protein carbonyl in the study group was significant increased (1.18-fold) than that of the control group ($p<0.001$). Whereas figure 2 shows the mean and standard deviation values of Sulphydryl protein content in the lens of normal control and experimental diabetic cataract. Protein sulphydryl content was significantly decreased (0.28-fold) in the study group as compared with control group ($p<0.001$).

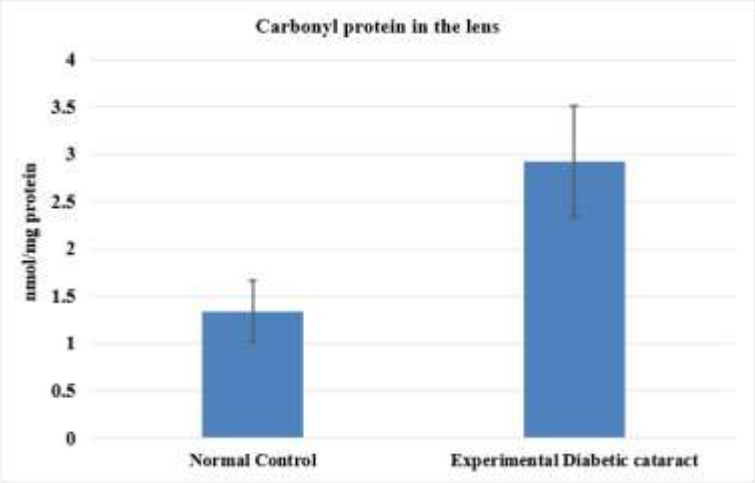

Figure 1: Carbonyl protein content in lens of normal Control and experimental diabetic cataract. Data are expressed as Means and SD. (p<0.001)

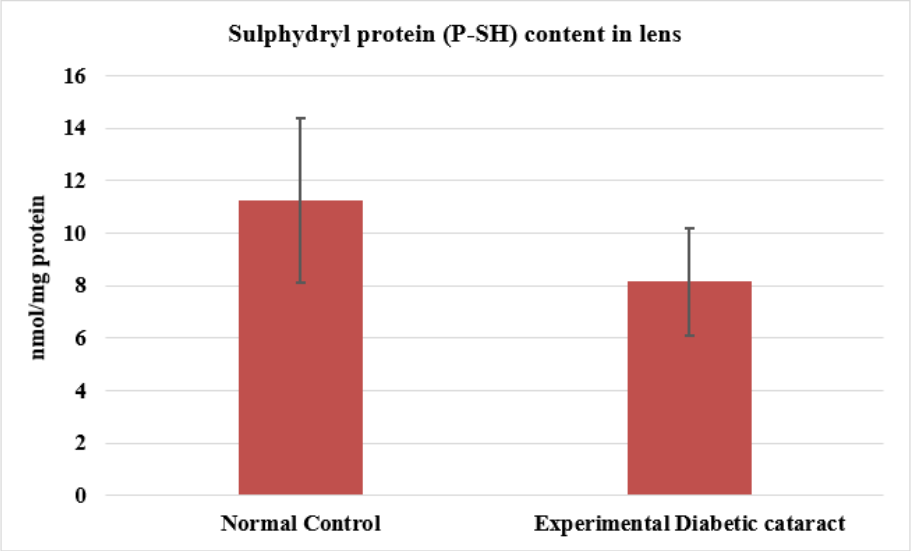

Figure 2. Sulfhydryl protein content in lens of Normal Control and experimental diabetic cataract. Data are expressed as Means and SD. (p<0.001)

Discussion

Diabetes can impact the eyes in a number of ways, but cataract is the most prevalent reason for vision loss. One of the early secondary consequences of diabetes mellitus, is cararactogenesis. From the results of our study, it has been clear that oxidation of lens protein plays an important role in the formation of cataract in the diabetic individuals. Protein carbonyl and protein sulphydryl came up with good biomarker of protein oxidation.

First biomarker, protein carbonyls are an irreversible kind of protein alteration that have been shown to be more durable than lipid peroxidation products, which are quickly destroyed. Additionally, protein carbonyls are a measure of total protein oxidation since they occur early under oxidative stress settings and are not the product of a single kind of oxidant [10]. It is known that diabetic patients' lenses contain higher levels of lipid oxidative products, which cause

further damage to several biomolecules, including proteins, and result in the formation of protein carbonyl [11]. Increased carbonyl synthesis in diabetes may be of significant relevance because increased oxidation of proteins results in the loss of their functions and may alter membrane permeability, which could further amplify the effect of cataract by increasing glycosylation of proteins.

The levels of sulfhydryl proteins reflect the reducing status of the proteins. The lens' capacity to maintain a reducing environment is undoubtedly crucial for cataract development and can have a big impact on when it starts. The lens proteins become more susceptible to sulfhydryl oxidation and the production of disulfide bonds as a result of either increased oxidative stress or the nonenzymatic glycosylation. It's likely that altering amino groups causes the protein molecule to shift shape, exposing sulfhydryl groups and aggregation of lens proteins[12].

In research conducted by Z. Kyseĺová et al. findings of protein oxidation such as protein carbonyl content were found to be significantly elevated whereas concentration of protein sulfhydryls were significantly decreased in experimental diabetic rats, which was in agreement with the findings of our study. This research was done on experimentally induced diabetes in rats using streptozotocin[13].

Our findings are found to be in concurrence with many other studies showing increased protein oxidation as one of the mechanism for pathophysiology of cataract[12,14,15].

Limitation of our study was that we only looked at indicators for protein oxidation. To better understand their function in redox status and cataract formation, additional characteristics such protein glycosylation and glutathione levels should be taken into consideration.

Conclusions

Taken together the above facts and results, the oxidation of lens protein in diabetic cataractogenesis seems to be strongly related. It has been hypothesized to involve the sulfhydryl oxidation, nonenzymatic glycosylation, and aggregation of lens proteins. Further The technique of lens organ culture adopted in this study was effective for examining in-vitro cataractogenesis's molecular basis and their prevention by using medicinal plant extracts.

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