Research Article

In vitro prevention of cataract by Oyster Mushroom *Pleurotus florida* extract on isolated goat eye lens

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ABSTRACT

Objectives: The aim of the present work was to evaluate the *in vitro* effect of *Pleurotus florida* extract cataract induced by glucose.

Materials and Methods: Goat eye lenses were divided into four groups. Group I lenses were incubated in artificial aqueous humor with glucose concentration 5.5 mM (normal control). Group II lenses were incubated with glucose concentration 55 mM (toxic control). Group III and IV lenses incubated with glucose concentration 55 mM were incubated along with hydroethanolic extract of *P. florida* 250 µg/ml and 500 µg/ml and subjected to morphological and biochemical evaluation.

Results: Group II lenses showed high amount of malondialdehyde (MDA) soluble and insoluble protein and decreased catalase and glutathione levels, while lenses treated with *P. florida* extract showed significant ($P < 0.05$) reduction in MDA, increased level of catalase ($P < 0.001$), glutathione ($P < 0.005$) and total and soluble protein.

Conclusions: Hydroethanolic extract of *P. florida* showed prevention of *in vitro* glucose induced cataract. Thus, the goat lens model could be used for testing of various anticataract agents.

KEY WORDS: Catalase, *in vitro,* glutathione, lens, *Pleurotus florida*

Introduction

Vision loss due to cataract is related to risk factors like malnutrition, sunlight, smoking, hypertension, aging, and diabetes.[1] Progress of cataract result into opaque eye lens leading to poor or complete vision loss.[2] Decrease in antioxidant enzyme activities in the cataractous lens points to the importance of antioxidant enzymes in the prevention of oxidative damage to the lens and subsequent development of cataract.[3] A wide range of drugs like aldose reductase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), etc have been tried for anticataract activity, but none are found to be effective.[4]

Mushrooms are considered as valuable health foods since they are low in calories and fats, and high in proteins and minerals.[5] Mushrooms are rich source of vitamins A, C β-carotene, and polyphenols.[6,7] *Pleurotus* sp. (Family: Pleurotaceae) is regarded as an edible mushroom for many years.[8,9] *P. florida* is also a rich source of phenolics and flavonoids.[10] *P. florida* possesses antioxidant, immunostimulator, antitumor, and anti-inflammatory activities.[11,12]

The aim of present work was to evaluate *in vitro* effect of *P. florida* on the development of cataract in goat eye lens model.

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Materials and Methods

Preparation of Extract

The mushroom basiciocarps were provided as gift sample from Professor Dr A.K. Pandey, Mycology Research Laboratory, Rani Durgavati University, Jabalpur (M.P.). The type specimen was deposited in Mycology Research Laboratory, Rani Durgavati University, Jabalpur (M.P.) (HDBJ#43). Mushrooms were dried in shade, coarsely powered, and used for preparation of extracts. The powder was extracted with ethanol: water (1:1) by stirring for 48 hrs and filtered through Whatman No. 4 filter paper. The residue was then extracted with two additional 200 ml portions of ethanol: water (1:1) as described above. The combined extracts was then evaporated at 40ºC to dryness and stored at 4ºC before further use.

Dose

The extract was used in the concentration of 250 μg/ml

and 500 μg/ml based on the previous study of *P. ostreatus*.[13]

Chemicals

Potassium chloride, sodium chloride, sodium bicarbonate, sodium phosphate, and calcium chloride were purchased from Central Drug House (CDH), India; glucose purchased from Fischer scientific (India), trichloroacetic acid, and ethylenediaminetetraacetic acid (EDTA) were purchased from Qualigens, India; thiobarbituric acid was purchased from Sigma, US. All other chemicals used were of analytical grade. Triple distilled water was used in the experiment.

Lens Culture

The study was carried out on goat lens due to easy availability from slaughter house. Fresh goat eyeballs were obtained from slaughter house and immediately transported to the laboratory at 0-4°C. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, $NaH(PO_4)_2$ 0.5 mM, $CaCl_2$ 0.4 mM and glucose 5.5 mM) at room temperature and pH 7.8 for 72 hrs. Penicillin 32 mg and streptomycin 250 mg were added to the culture media to prevent bacterial contamination.[14]

Induction of in vitro Cataract

Glucose in a concentration of 55 mM was used to induce cataract.[14] At high concentrations, glucose in the lens metabolizes through sorbitol pathway and accumulation of polyols (sugar alcohols) causing over hydration and oxidative stress. This led to cataractogenesis. A total of 24 lens were used for the study. These lenses were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM served as normal control and 55 mM served as toxic control) for 72 hours.

Study Design and Groups

Goat lenses were divided into four groups of six lens each and incubated as follows:

Group I: Glucose 5.5 mM(normal control);

Group II: Glucose 55 mM(toxic control);

Group III: Glucose 55 mM + *P. florida* extract 250 μg/ml; and

Group IV: Glucose 55 mM + *P. florida* extract 500 μg/ml.

Protein Estimation

For total protein estimation, the lens homogenate was prepared in 5% trichloroacetic acid. The precipitated protein was dissolved in sodium hydroxide and aliquots were used for the estimation of total proteins. Soluble and insoluble fractions of the protein were estimated by preparing homogenate in double distilled water. The water soluble supernatant was used for estimation of soluble protein and the residue was dissolved in sodium hydroxide and used for the estimation of insoluble protein. The protein content of the samples was determined by the method of Lowry *et al.*[15] using bovine serum albumin as the standard.

Biochemical Estimation

Glutathione estimation was done as reported by Ellman.[16] Lens catalase activities were determined by Goth's colorimetric method.[17] Lipid peroxide (malondialdehyde) formed was estimated by measuring thiobarbituric acid reacting substances (TBARS).[18]

Morphological and Photographic Evaluation

Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of squares clearly visible through the lens) was observed to measure lens opacity. The degree of opacity was graded as follows:

0 : Absence

\+ : Slight degree

++ : Presence of diffuse opacity

+++ : Presence of extensive thick opacity

Statistical Analysis

All data were expressed as mean ± SD. The groups were compared using one-way ANOVA with post-hoc Dunnett's test using glucose 55 mM group as control. $P < 0.05$ was considered significant.

Results

Protein Content

Group II showed significant decrease in lens protein level ($P < 0.005$) as compared to group I. *P. florida* extract treated groups III and group IV showed significant increase ($P < 0.05$) in lens protein as compared to group II [Table 1].

Lens Glutathione Level

Group II showed significantly ($P < 0.005$) less glutathione level as compared to normal control group I. *P. florida* extract at the concentration of 250 μg/ml and 500 μg/ml showed significant increase ($P < 0.05$) in lens glutathione as compared group II.

Lens Catalase Levels

Incubation of lens in glucose resulted in a time dependent inactivation of the enzymes. Lens catalase activities were significantly lower in group II as compared to *P. florida* extract treated groups [Table 1].

Table 1:

Effects of *P. florida* extract on antioxidant enzymes, MDA, and proteins on isolated goat lens model

Parameters	Group I (Glucose 5.5 mM)	Group II (Glucose 55 mM)	Group III (Glucose 55 mM + P. florida 250 mcg/ml)	Group IV Glucose 55 mM+ P. florida 500mcg/ml))
Total protein (mg)	195.5±0.2**	160.2±7.7	171.8±5.0*	187.1±2.6*
Water soluble protein (mg)	93.3±0.4***	74.9±3.4	87.4±2.1*	89.9±2.2*
Glutathione (μmoles/g)	7.9 ±0.1**	5.7±0.2	6.3±0.1**	7.1±0.1**†
Catalase (kU/l)	8.4±0.1***	3.9±0.1	6.3±0.1***	7.7±0.2***†
MDA (nmoles /mg)	3.0±9.9*	60.7±17.4	47.1±8.3*	29.7±6.9*

Values are mean±SD, n = 6; MDA= Malondialdehyde; *$P < 0.05$, **$P < 0.005$ and ***$P < 0.001$ as compared with their corresponding value in group II; †$P < 0.005$ as compared with group III

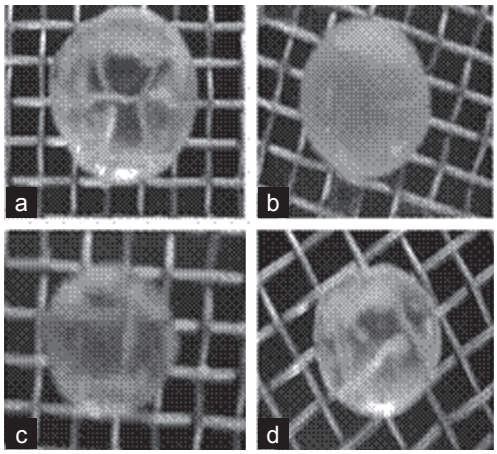
MDA Levels

MDA levels were found to be high in group II as compared to group I (normal lens). Lenses treated with *P. florida* extract had significantly ($P < 0.05$) reduced MDA content at both concentrations compared with high glucose group [Table 1]. The level of lipid peroxides was expressed as nmoles of MDA formed/mg protein.

Lens Morphology in vitro/ Photographic Evaluation

All six lenses in group I remained transparent while all six lenses in group II developed dense opacities. The opacity progressively increased towards centre with complete opacification at the end of 72 hours. While *P. florida* extract at 250 μg/ml and 500μg/ml retarded the development of opacity. The grades of opacity was 0, +++, ++ and + in group I, II, III and IV, respectively. In group III, four out of six lens; and in group IV, five out of six lens the changes were observed [Figure 1].

Figure 1: (a) Group I: Normal lens after 72 hours of incubation in glucose 5.5 mM (Transparency maintained, squares clearly visible). (b) Group II: Complete cataractogenesis after 72 hours of incubation in glucose 55 mM (Absolute loss of transparency, no squares visible through lens). (c) Group III: After 72 hours of incubation in glucose 55mM + Extract 250 µg/ml, lens appears slightly hazy (Very less no. of squares slightly visible). (d) Group IV: After 72 hours of incubation in glucose 55mM + Extract 500 µg/ml, lens appears slightly hazy (More no. of squares visible)



Discussion

Cataract is one of the universal processes of ageing and is consequence of cumulative effect of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species play an important role in the process leading to lens opacification.[19] This oxidative crisis is one of the reasons for generation of cataract.[20]

Cataract related studies on animal models are laborious and time consuming.[21] However, *in vitro* model for inducing cataract using glucose concentration 55 mM provides an effective model on isolated lenses of mice[13,20] and goat[14] etc.

Catalase is an important part of the innate enzymatic defense system of the lens which is responsible for the detoxification of H_2O_2. Decrease in the activities of these enzymes in tissues has been linked with the buildup of highly reactive free radicals leading to injurious effects such as loss of integrity and function of cell membranes.[22] In this study, the level of catalase was found to be less in toxic control lens as compared to normal control group. The lenses treated with *P. florida* showed significant rise in enzyme level suggesting maintenance of antioxidant enzyme integrity.

The amount of reduced glutathione in the lens decreases in almost in any type of cataract.[4,23] The role of reduced glutathione in the preservation of lens clarity is of substantial interest; it serves as the major antioxidant in the lens and prevents protein oxidation.[23] The restoration of reduced glutathione levels by *P. florida* extract also demonstrated its anticataract potential.

Under stressful condition, the protein of the lens denatures and creates disulfide cross linking causing disulfide and mixed disulfide bond formation, causing protein aggregation, precipitation leading to lens opalescence.[24] However, *P. florida* treatment increased the protein level in lens. In this study, the levels of MDA were more in toxic control group as compared to group I, III, and IV suggestive of preventive role of *P. florida* extract against *in vitro* glucose induced cataract. In addition, *P. florida* extract was able to retard *in vitro* glucose induced cataract. This study shows that antioxidant enzymes like catalase and glutathione protects the eye lens against oxidative damage.

Hence, it can be concluded that oxidative stress is an important factor in the development of cataracts and the use of antioxidants[25] may be advocated in patients to delay or prevent formation of cataract. *P. florida* showed protective *in vitro* activity against glucose cataract in an isolated goat lens model. This effect may be attributed due to maintenance of higher levels of protective antioxidant enzymes as well as water soluble protein in the lens.

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