**Research Article**

**In-vitro Prevention of Cataract by Ginseng on Isolated Goat Eye Lens**

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**ABSTRACT:** Objective: The aim of the present work was to evaluate the in vitro effect of ginseng on 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 ginseng 50 and 100mg/ml and subjected to morphological and biochemical evaluation. Results: Group II lenses showed high amounts of malondialdehyde (MDA) soluble and insoluble protein and decreased catalase and glutathione levels. Lenses treated with ginseng showed significant (P < 0.05) reductions in MDA, as well as increased levels of catalase (P < 0.001), glutathione (P < 0.005) and total and soluble protein. Conclusions: Ginseng showed prevention of in vitro glucose induced cataract. Thus the goat lens model could be used for testing of various anticataract agents.

KEY WORDS: Cataract, catalase, in vitro, glutathione, lens, ginseng, *Panax ginseng*

**INTRODUCTION**

Vision loss due to cataract is related to risk factors including malnutrition, sunlight, smoking, hypertension, aging, and diabetes[1]. Progression of cataracts results in opaque eye lenses leading to poor or complete vision loss[2]. Decreases 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 the subsequent development of cataract[3]. A wide range of drugs including aldose reductase inhibitors, non-steroidal anti-inflammatory drugs (NSAIDs), etc have been trialled for anticataract activity, however none are found to be effective[4].

*Panax ginseng*, also known as Korean or Chinese ginseng, has been used as a general tonic in traditional oriental medicine to increase vitality, health, and longevity, especially in older person[5]. Pharmacological effects of ginseng have been demonstrated in the CNS as well as in cardiovascular, endocrine, and immune systems. In addition, ginseng has been ascribed to possess antineoplastic, antistress, and antioxidant activity[6]. Evidence pointing to the medical efficacy of ginseng has been closely linked to its protective properties against free radical attack[7]. *Panax ginseng* administration in rats prevents myocardial ischemia-reperfusion damage induced by hyperbaric oxygen[8] and was reported to have a hepatoprotective effect on oxidative stress induced by exhaustive exercise[9]. Ginseng protects smokers from oxidative damage and reduces the cancer risk associated with smoking[10]. Ginseng was reported to scavenge hydroxyl[11] and superoxide radicals[12]. Ginseng was also reported to inhibit lipid peroxidation through transition metal chelation[13] to diminish oxidative DNA damage caused by fenton reagent or UV exposure. Ginseng protected human low density lipoproteins against oxidation in vitro[14,15] and induced Cu2+, Zn2+–superoxide dismutase expression at the transcriptional level[16]. The aim of present work was to evaluate in vitro effect of ginseng the development of cataract in goat eye lens model.

**MATERIALS AND METHODS**

**Ginseng**

Ginseng powder was obtained from Sai Mirra Innopharm, Chennai. Potassium chloride, sodium chloride, sodium bicarbonate, sodium phosphate, and calcium chloride
were purchased from Central Drug House (CDH), India of analytical grade; glucose was 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 obtained from local slaughter house. Fresh goat eyeballs were obtained from the 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 5mM, MgCl2 2 mM, NaHCO3 0.5 mM, NaF (PO4)2 0.5 mM, CaCl2 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[17].

**Induction of in-vitro Cataract**

Glucose at a concentration of 55 mM was used to induce cataracts.[17] At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataractogenesis.[17] A total of 24 lenses 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 + ginseng 50 mg/ml; and Group IV: Glucose 55 mM + ginseng 100 mg/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 the homogenate in double distilled water. The water soluble supernatant was used for the 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[18] using bovine serum albumin as the standard.

**Biochemical Estimations**

Glutathione estimation was done as reported by Ellman[19]. Lens catalase activities were determined by Goth’s colorimetric method[20]. Lipid peroxide (malondialdehyde) formed was estimated by measuring thiobarbituric acid reacting substances (TBARS)[21].

**Morphological and Photographic Evaluation**

Lenses were placed on a wired mesh with the posterior surface touching the mesh. 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 (Table 1). Ginseng treatment (groups III and group IV) showed significant increases (P < 0.05) in lens protein as compared to group II.

**Lens Glutathione Level**

Group II showed significantly (P < 0.005) less glutathione compared to the normal control group I (Table 1).
Ginseng at the concentrations of 50 mg/ml and 100 mg/ml showed significant increases (P < 0.05) in lens glutathione as compared group II.

**Lens Catalase Levels**

Incubation of lens in glucose resulted in inactivation of the enzymes. Lens catalase activities were significantly lower in group II as compared to ginseng treated groups (Table 1). These levels increased closer to the control levels with ginseng treatment.

**MDA Levels**

MDA levels were found to be high in group II (high dose) as compared to group I (normal control lens). Lenses treated with ginseng had significantly (P < 0.05) reduced MDA content at both concentrations compared with high glucose group (Table No 1).

**Lens Morphology in vitro/ Photographic Evaluation**

All six lenses in group I remained transparent whilst all six lenses in group II developed dense opacities (figure 1). The opacity progressively increased towards the centre with complete opacification by 72 hours. Ginseng at 50 mg/ml and 100mg/ml retarded the development of opacity compared to group II. 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 showed the reported changes.

**DISCUSSION**

Cataract is one of the universal processes of ageing and is a consequence of cumulative effects of various insults to the lens. The oxidation of lens proteins by free radicals and reactive oxygen species plays an important role in the process leading to lens opacification[22]. This oxidative crisis is one of the reasons for the generation of cataract[23]. Cataract related studies on animal models are laborious and time consuming[24]. However, the in vitro model for inducing cataract using glucose concentration 55 mM provides an effective model on isolated lenses of mice[23] and goat[17].

Catalase is an important part of the innate enzymatic defense system of the lens which is responsible for the detoxification of H₂O₂. Decreases in the activities of these enzymes 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[25]. In this study, the level of catalase was found to be less in the toxic control lens as compared to normal control group. The lenses treated with ginseng showed a 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[6,29]. 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[29]. The restoration of reduced glutathione levels by ginseng supports its anticataract potential. Under stressful condition, the protein of the lens denatures and creates disulfide cross linking and mixed disulfide bond formation. This causes protein aggregation and, precipitation leading to lens opalescence[27]. However, ginseng treatment increased the protein level in lens. In this study, the levels of MDA were more in the toxic control lens as compared to group I, III, and IV suggestive of a preventive role of ginseng against in vitro glucose induced cataract. In addition, ginseng was able to retard in vitro glucose induced cataract. This study indicates that the antioxidant enzymes including catalase and glutathione protects the eye lens against oxidative damage.

**CONCLUSION**

Oxidative stress is an important factor in the development of cataracts and the use of antioxidants[28] may be advocated in patients to delay or prevent the formation of cataracts.
of cataract. Ginseng showed protective in vitro activity against glucose induced cataract in an isolated goat lens model. This effect may be attributed to the maintenance of higher levels of protective antioxidant enzymes, as well as water soluble protein in the lens.

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REFERENCES