ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5):1265-1270

ORIGINAL RESEARCH ARTICLE

Berberine from roots of *Berberis aristata* prevents cataract formation in isolated goat eye lens: An *In-vitro* Study

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Received 02 Jun 2012; Revised 25 Sep 2012; Accepted 10 Oct 2012

ABSTRACT

The aim of this study was to evaluate *In-vitro* efficacy of berberine isolated from roots of *Berberis aristata* in isolated goat lens. The goat lenses were incubated in aqueous solution of glucose in 55mM concentration for 72 hrs. The lenses were divided in to four groups; Group I served as toxic control and Group II, III, IV served as berberine treated (1, 2 and 5 mg/ml). Group I showed significant increase in MDA levels along with decreased catalase and glutathione levels. The berberine treated groups showed significant increase ($P<0.001$) in glutathione, catalase and protein levels. Thus isolated berberine showed protective effects against *In-vitro* glucose induced models and can be used as an anticataract agent.

Keywords: Berberine; cataract; goat; eye; lens

1. INTRODUCTION

A cataract is a clouding that develops in the crystalline lens of the eye or in its envelope, varying in degree from slight to complete opacity and obstructing the passage of light. (1)Cataracts are clumps of protein that collect on the lens of an eye and interfere with vision. Normally, light passes through the lens (the clear tissue behind the pupil) and focuses on the retina.(2, 3) Oxidative damage by the free radicals is also implicated in the Pathology of catarectogenesis.(4) generally cataract formation is caused by polyol pathways glycation within lens fibers and the epithelium. Sugar can passively diffuse into lens tissue and then can be converted by aldose reductase to polyol, which can't diffuse passively out of lens, and thus they accumulate. This accumulation causes osmotic changes, which leads to lens hydration and swelling that are followed by biochemical and physiological damage to cell membrane. (5)

"Ayurveda" is a traditional medicine system which was developed during the Vedic times, around 5000 years ago.(6) One of the traditional medicinal plants from ayurvedic system is known as *Berberis aristata* (Daruharidra) and its herbal formulations are used to treat malaria, bleeding, fever, skin and eye infections, jaundice, diarrhoea and hepatitis for a long time. Its traditional use as anti-microbial, anti-bacterial, anti-pyretic, immunostimulant, laxative, anti-haemorrhagic and anti-inflammatory agent is also well known. The plant contains a number of important phytochemicals which are alkaloids of the type proto-berberine, isoquinoline, bisbenzyl-isoquinoline and other bioactive constituents like flavonoids and phenolic acids.(7,8,9)

The formulations called 'Netrabindu', 'M.D.H drops and capsules' and 'Madhudarvyadi eye drops' containing *B. aristata* are used for treatment of conjuctivitis (10) and similarly an ayurvedic formulation containing this plant is used to treat complications of cataract (11). Ayurvedic preparation which is Anjana called Elanir kujambu contains B. aristata. It is a good medicine for treatment of eye diseases and infections because of its anti-microbial and anti-bacterial activity. (12)

Since there are no scientific reports on *In-vitro* effects of *B. aristata* on the development of

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cataract in goat eye lens models. Berberine is the main active constituents of roots and present in very higher amount, thus in present study, we examined the inhibitory effects of isolated berberine in whole lens cultures in goat lens.

2. MATERIALS AND METHODS

2.1 Plant Materials-

Dried roots of *Berberis aristata* were purchased from local market and authentified by Dr. Gyanendra Tiwari (Head, Dept of Botany, KNK college of Horticultures, Mandsaur).

2.2 Preparation of Extract and Isolation of Berberine-

The dried roots were extracted by ethanol using soxhlet apparatus. The extract is dried under vacuum and residue is dissolved in hot water and solution is filtered. He filtrate is treated with excess amount of hydrochloric acid and allowed to stand for some time and the crystals of berberine hydrochloride are separated out.

2.3 Purification of isolated berberine hydrochloride-

The salts of berberine are dissolved in hot water and the solution is made alkaline with few drops of 10% NaOH. Thereafter a small quantity of acetone is added and solution is diluted with equal amount of water. The precipitate is allowed to settle overnight and washed with ice-cold water. The structure of isolated berberine is determined on the basis of TLC, IR & NMR. (13)

2.4 Thin Layer Chromatography of isolated Berberine-

For the TLC identification precoated silica gel plates were used. Ethyl acetate, acetone, formic acid and water is used as developing solvents. (13) (table 1)

Table I: Solvent systems for TLC Study

S. No	Drug	Solvent system	Rf value
1	Berberine Std	Ethyl Actate : Acetone : Formic Acid : Water (100 : 11 : 11 : 27)	0.75
2	Test Drug	Ethyl Actate : Acetone : Formic Acid : Water (100 : 11 : 11 : 27)	0.75

2.5 Chemicals-

Potassium chloride, sodium chloride, sodium bicarbonate, sodium phosphate and calcium chloride, EDTA, trichloroacetic acid was procured from central drug sore of MIP, Mandsaur. All the chemicals used were of analytical grade. The triple distilled water was used in the experiments.

2.6 Lens cultures-

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 0-4ºC. The lenses were removed by extracapsular extraction and incubated in aqueous humor at room temperature and pH 7.8 for 72 hrs. Penicillin 32 mg was added to he culture media to prevent bacterial contaminations. (14)

2.7 Induction of *In-vitro* Cataract-

Glucose in a concentration of 55mM was added to induce cataract. At higher concentration, glucose in lens metabolizes through sorbitol pathways and accumulation of polyol (sugar alcohols) causing over hydration and oxidative stress & leads to cataractogenesis. These lenses were incubated in aqueous humor for the 72 hrs.

2.8 Study design and Groups-

Goat lenses were divided into 4 groups of six lenses each and incubated as follows.
Group-I: Glucose 55 mM (Toxic control)
Group-II: Glucose 55 mM + Berberine 1 mg/ml
Group-III: Glucose 55 mM + Berberine 2 mg/ml
Group-IV: Glucose 55 mM + Berberine 5 mg/ml

2.9 Biochemical Estimation-

The levels of sodium and potassium were measured by flame photometry. Glutathione estimation was done as reported by Ellman methods (15), protein estimation by Lowry *et al* (16), catalase by Goths mehod (17), lipid peroxidation by TBARS (18).

2.10 Morphological and Photological Evaluation-

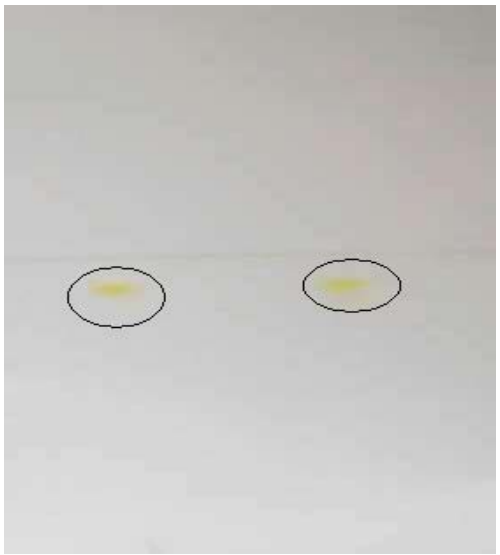
Lenses were placed on a wired mesh with posterior surface touching mesh, and the pattern of the mesh (number of squares clearly visible through lens) was observed to measure lens opacity. The degree of opacity was graded as follows:
0: Absence
+: Slight degree
++: Presence of diffuse opacity
+++: Presence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

2.11 Statistical analysis-

All the data were expressed as mean±SEM. The groups were compared using one-way ANOVA with dunnetts test using glucose 55mM group served as control. P<0.05 was considered significant.

3. RESULTS

3.1 TLC Characterization of isolated berberine with standard



(S) (T)

Fig No. 1- TLC of Test & Std. Berberine sample

3.2Sodium and Potassium Levels-

All the berberine treated groups showed significantly decreased (P<0.01) levels of sodium as compared to group I and increased levels (P<0.01) of potassium when compared to group I (Toxic control).

3.3 Protein content-

Group III and IV showed significant increase (P<0.01, 0.001) in protein content as compared to group I.

3.4 Glutathione content-

Group I showed significantly decrease in glutathione level. The berberine treated groups (III and IV) showed significantly increase (P<0.01, 0.001) in glutathione levels.

3.5 Catalase levels-

Incubation of lens in glucose solution causes inactivation of various enzymes. In glucose treated lens 55mM showed significant decrease in catalase levels. While berberine treated groups showed significant increase in catalase levels (P<0.01) when compared to Group I.

3.6 MDA levels-

The berberine treated lens had showed significant decrease in lipid peroxidation levels when compared to glucose 55 mM treated lens. The level of MDA was expressed in nmoles of MDA formed/mg protein.

3.6 Lens morphological evaluation-

All the lens of group I developed dense opacities. The opacity was progressively increased towards centre with complete opacification at the end of 72 hrs. While berberine treated at 1, 2 and 5 mg/ml retarded the development of opacity. The grades of opacity was +++, ++, +.

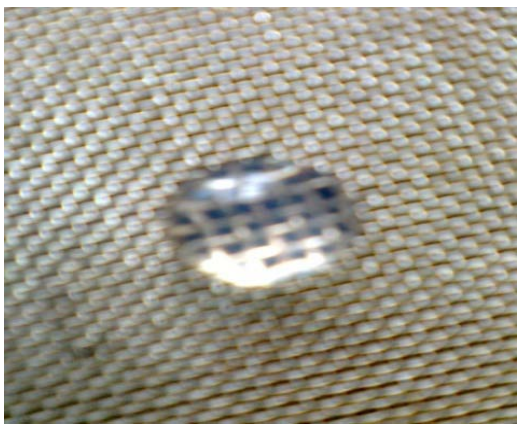


Fig-2 Normal lens before 72 h of incubation.

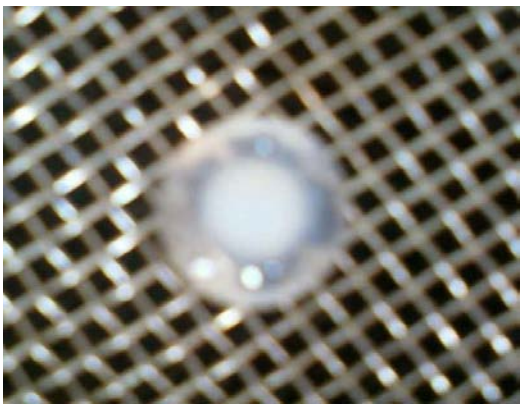


Fig-3 Lens after 72 h of incubation with glucose 55 mM.

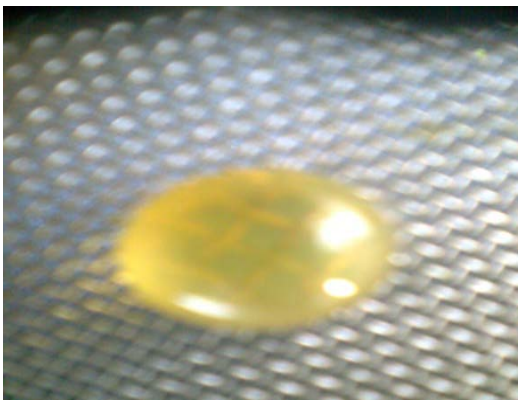


Fig-4 Lens after 72 h of incubation with glucose 55 mM+ berberine 2 mg/ml.

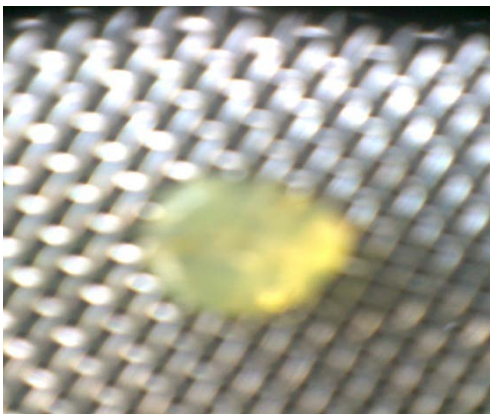


Fig-3 Lens after 72 h of incubation with glucose 55 mM+ berberine 1 mg/ml.

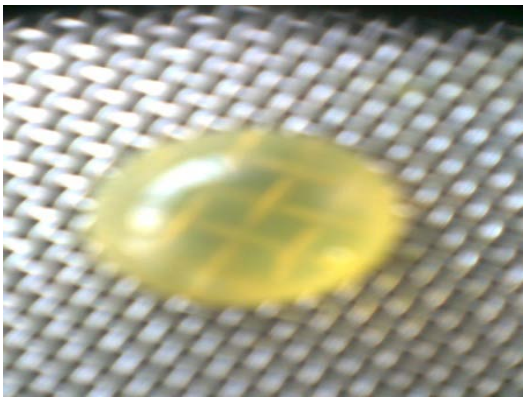


Fig-5 Lens after 72 h of incubation with glucose 55 mM+ berberine 5 mg/ml.

Table No. II- Effect of berberine on Na^+, K^+ activity in lens homogenate after 72 hrs of incubation

S. No.	Groups	Sodium content	Potassium content
1	Control (Glucose 55 mM)	107.8±0.7032***	8.317±0.1195***
2	Glucose 55 mM + berberine 1 mg/ml	96.00±0.5774*	9.450±0.07638
3	Glucose 55 mM + berberine 2 mg/ml	88.50±0.9916**	10.12±0.1078**
4	Glucose 55 mM + berberine 5 mg/ml	76.17±0.4773***	11.50±0.1515***

Table No. III- Effect of berberine on protein in lens homogenate after 72 hrs of incubation

S. No.	Groups	Total Protein (mg)	Water Soluble Protein (mg)
1	Control (Glucose 55 mM)	158.2±7.4	73.8±2.1
2	Glucose 55 mM + berberine 1 mg/ml	160.4±0.4	78.9±0.21*
3	Glucose 55 mM + berberine 2 mg/ml	164.8±0.65**	82.7±1.24**
4	Glucose 55 mM + berberine 5 mg/ml	180.8±1.3***	90.1±0.62***

Table No. IV- Effect of berberine on glutathione content of goat eye lenses kept under glucose induced cataract

S. No.	Group	Glutathione content (mg/ml of lens homogenate)
1	Control(glucose 55 mM)	0.2262±0.001302
2	Glucose 55 mM + berberine 1 mg/ml	0.2368±0.001376*
3	Glucose 55 mM + berberine 2 mg/ml	0.2508±0.0007923**
4	Glucose 55 mM + berberine 5 mg/ml	0.2710±0.0020***

Table No. V- Effect of berberine on catalase and MDA in lens homogenate after 72 hrs of incubation

S. No.	Groups	Catalase (kU/l)	MDA (nmoles/mg)
1	Control (Glucose 55 mM)	3.7±0.21	58.8±1.4
2	Glucose 55 mM + berberine 1 mg/ml	3.9±0.84	55.5±0.45*
3	Glucose 55 mM + berberine 2 mg/ml	4.3±0.36**	50.3±0.12**
4	Glucose 55 mM + berberine 5 mg/ml	7.8±0.68***	42.8±0.64***

4. DISCUSSION

Diabetic cataract is a late stage complication in diabetic patients and majorly affects the quality of life. There are few drugs are available for the treatments of diabetic cataract and those have a great target on aldose reductase, a key enzymes in polyol pathway of sugar metabolism. (19) the osmotic stress theory can still be applied in humans since sorbitol accumulation in diabetic patients to cause osmotic damage that raised increased aldose reductase activity and leads to diabetic complication such as cataract. (20)

Since cataract studies on animal models are very laborious and time consuming so In-vitro models for the induction of cataract using higher concentration of glucose is an very effective model in isolated mice or goat lenses.

In our body glutathione serves as a protective role in various diseases and reduced glutathione in prevention of lens clarity. It serves as a potent antioxidant and prevents protein oxidation. The restoration of reduced glutathione levels by berberine demonstrated its anticataract property.

Catalase is important enzymes in defense system of lens and decreased concentration of it causes buildup of highly reactive free radicals leads to injurious effects like loss of integrity and function of cell membranes. In this study, the lens treated with berberine showed significant rise in enzyme and suggesting its maintenance of antioxidant enzyme integrity.

Under stress full condition, the protein of lens denatures and causing aggregation of protein and this precipitation leads to opacification of lens. However, the treatments of berberine causes increased levels of proteins in lens. In berberine treated groups, MDA levels were significantly decreased when compared to glucose 55mM treated groups. The alteration in sodium and potassium levels causes decrease in water soluble proteins and increase in insoluble proteins. This causes lens opacification

This study shows that antioxidant enzymes like catalase and glutathione protects the eye lens against oxidative damage.

5. CONCLUSION

Berberis aristata showed protective *In-vitro* activity against glucose cataract in an isolated goat lens models. These effects may be attributed due to maintenance of higher levels of protective antioxidant enzymes as well as protein levels in eye lens.

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