

Evaluation of Echitamine, Ditamine and Echitenine for Anticataract Activity using in vitro cataract Model

Lokesh J Shetty

Department of Pharmacology and Toxicology, Riyadh Colleges of Dentistry and Pharmacy. P O Box 84891, Riyadh 11681, Saudi Arabia.

ARTICLE INFO

Article history:

Received on: 19/11/2014

Revised on: 02/02/2015

Accepted on: 07/04/2015

Available online: 27/05/2015

Key words:

Anti- cataract, Echitamine, ditamine, echitenine.

ABSTRACT

The alkaloids such as echitamine, ditamine and echitenine were subjected for anti cataract activity using *in vitro* cataract model. Goat lenses were incubated in artificial aqueous humor containing 55 mM glucose with test alkaloids in different concentrations at room temperature for 72 h. Studied Biochemical parameters were sodium ion, potassium ion level and Na⁺-K⁺-ATPase activity. Glucose induced opacification of goat lens began 8-10 hrs after incubation and was complete in 72-80 hrs. Cataractous lenses treated with echitamine shown lower sodium ion, higher the potassium ion and higher Na⁺-K⁺-ATPase activity. Echitamine treated lens prevented formation and progress of cataract as evidenced by biochemical parameters and visual observation. Ditamine and echitenine were devoid of anti - cataract activity.

INTRODUCTION

Cataract, the clouding of the lens develops later in the life and it is most likely the consequence of decades of accumulated damage to long lived lens protein. It is the most common cause of blindness. Visual loss occurs because of opacification of the lens obstructs light from passing and being focused onto the retina (Klein *et al.*, 1997, Kinoshita.,1974, Quillen., 1999). It occurs mainly due to the formation of large protein aggregates in the lens. The research has shown that post translational modification of the lens crystallins such as oxidation, glycation, carbamylation, transamidation, phosphorylation and proteolysis lead to opacification of lens (Kinoshita., 1974, Harding and Rixon., 1980). Oxidative mechanism plays an important role in biological phenomena, including cataract formation. The formation of superoxide radicals in the aqueous humor and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to opacification (Harding and Rixon., 1980). The aldose reductase enzyme also plays an important role in pathogenesis of cataract. The aldose reductase acts on glucose, galactose and xylose and

convert them into their respective alcohols. These alcohols, also known as polyols accumulate within the lens there by producing osmotic effects. Since polyols are not capable of diffusing out easily nor metabolizes rapidly and causes hyper tonicity responsible for opacification (Kinoshita *et al.*, 1962). The higher glucose level also leads to the formation of superoxide radical and Hydrogen peroxide (Ceriello *et al.*, 1996). The research is also implicated that Na⁺-K⁺-ATPase activity is important in maintaining ionic equilibrium in the lens, and its impairment causes accumulation of sodium ion and loss of potassium ion with hydration and swelling of the lens fibers leading to cataractogenesis (Chakravarthi *et al.*, 1968). There has been no scientifically proven means of preventing cataracts. While it had been thought that regular intake of antioxidants would protect against the risk of development of cataracts, clinical trials have shown that their use as a supplement does not (Mathew *et al.*, 2012). On the other hand, research is mixed, but weakly positive, for a potential protective effect of the nutrients such as lutein and zeaxanthin (Barker, 2010). There are no any prophylactic approaches are available to prevent cataract formation, none evaluated found to be effective. In the present study, we evaluated alkaloids such as echitamine, ditamine and echitenine for anti cataract activity using in vitro cataract model. These alkaloids are abundantly found in stem bark of plant *Alstonia scholaris* belongs to the family Apocynaceae (Kapoor., 2001). At present these alkaloids are available from commercial suppliers.

* Corresponding Author

LOKESH J SHETTY, Department of Pharmacology and Toxicology/ Riyadh Colleges of Dentistry and Pharmacy. P O Box 84891, Riyadh 11681, Saudi Arabia. lokesh.shetty@riyadh.edu.sa

MATERIALS AND METHODS

Chemicals and Drugs

The chemicals and reagents used in the present study were of analytical grade.

Eye balls Collection & Lens culture Preparation

The study was approved by Institutional Animal Ethics Committee. Goat eye balls were used in present study, were obtained from the slaughterhouse immediately after slaughter and transported to the laboratory at predefined condition of 0-4 degree Celsius. The lenses were removed by an extra capsular extraction method and incubated in artificial aqueous humor (NaCl 140 mM, KCl 55 mM, MgCl₂ 2 mM, NaHCO₃ 0.5 mM, NaH(PO₄)₂ 0.5 mM, CaCl₂ 0.4 mM and Glucose 5.5 mM) at room temperature for 72 h. The pH of the culture is maintained at 7.8. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent lens deterioration by bacterial contamination. Glucose in a concentration of 55 mM was used as an inducer of cataract (Chandrokar *et al.*, 1981).

Test drug concentration and Groups & Biochemical estimation

Three concentrations of the test drug were chosen from test drug category. Test concentrations were chosen in such a way that the middle dose as 5mg/ml, high dose which was twice that of middle, and the lowest was 50% of middle concentration. A total of 66 lenses were divided into following categories in such a way that each group contains 6 lenses.

Group I: Normal lens [Control (Glucose 5.5mM)]

Group II: Glucose 55mM

Group III

E1: Echitamine 5 mg/ml of lens culture + Glucose 55 mM

E2: Echitamine 2.5 mg/ml of lens culture + Glucose 55 mM

E3: Echitamine 10 mg/ml of lens culture + Glucose 55 Mm

D1: Ditamine 5 mg/ml of lens culture + Glucose 55 mM

D2: Ditamine 2.5 mg/ml of lens culture + Glucose 55 mM

D3: Ditamine 10 mg/ml of lens culture + Glucose 55 mM

C1: Echitenine 5 mg/ml of lens culture + Glucose 55 mM

C2: Echitenine 2.5 mg/ml of lens culture + Glucose 55 mM

C3: Echitenine 10 mg/ml of lens culture + Glucose 55 Mm.

After 72 h of incubation, Lenses were placed on a wired mesh with the posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity. Homogenate of lenses was prepared then in Tris buffer (0.23M, pH 7.8) containing 0.25X10⁻³ sub M EDTA and homogenate adjusted to 10 % w/v. The homogenate was centrifuged at 10,000 G at 4°C for 1 hour. The supernatant obtained used for estimation of biochemical parameters. Electrolyte (Na⁺ & K⁺) estimation was done by flame photometry. Na⁺-K⁺-ATPase activity was assessed by the method of Unakar & Tsui method (Unakar and Tsui., 1980, Chylack and Kinashita., 1969).

Statistical analysis

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

RESULTS

Visual evaluation shown that the Group II containing glucose 55 mM and Echitenine and Ditamine treated group in all concentration of Group III, has shown opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the center, with complete opacification observed at the end of 72 hrs. Group I, treated with 5.5 mM of glucose and Echitamine treated group, Group IIIE1 and Group IIIE3 retarded the progression of lens opacification, compared with Group II.

Group II has shown significantly higher sodium ion (P<0.05), lower potassium ion (P<0.001) and lower Na⁺-K⁺-ATPase activity (P<0.001) compared with Group 1. [Table 1] Group IIIE1 and E3 has shown significantly high potassium ion (P<0.001), while Na⁺-K⁺-ATPase activity was significantly higher (P<0.001) in Group IIIE3, mine compared with Group II. Group IIIE3 has shown significant decrease in sodium ion (P<0.05). The results of Echitenine and Ditamine treated groups were statistically non significant at all doses.

Table 1: Na⁺, K⁺ and Na⁺-K⁺-ATPase activity in lens homogenate after 72 h of incubation.

Study Groups		Na ⁺ [meq/gm]	K ⁺ [meq/gm]	Na ⁺ -K ⁺ -ATPase activity [μgP/gm]
Group I	Control	53.9 ± 4.1**	10.5 ± 1.5***	53.9 ± 2.1***
Group II	Glucose 55mM	214.7 ± 9.7	6.7 ± 0.5	14.7 ± 2.9
Group III	E1	182.2 ± 23.6	8.9 ± 0.7**	38.4 ± 2.3**
	E2	202.4 ± 13.5	6.9 ± 0.7	22.3 ± 3.1
	E3	132.3 ± 12.7*	9.6 ± 1.2***	46.1 ± 3.1***
	D1	205.7 ± 13.5	6.2 ± 0.4	13.8 ± 2.2
	D2	217.4 ± 21.5	7.1 ± 0.4	14.3 ± 3.9
	D3	221.6 ± 10.7	6.1 ± 0.2	13.7 ± 4.2
	C1	201.6 ± 14.2	6.2 ± 0.3	15.7 ± 4.6
	C2	213.4 ± 19.5	6.9 ± 0.5	17.7 ± 3.5
	C3	214.3 ± 14.5	6.7 ± 0.8	18.7 ± 5.9

n= 6 in each category.

DISCUSSION

Hydration is the main reason for the development of cataract (Kinoshita., 1965). The striking osmotic imbalance due to an influx of sodium and chloride ions is apparent in cataractogenesis. It becomes obvious that one important mechanism in the lens is that which maintains the normal state of hydration.

The lens volume is a balance of two opposing forces: one is the normal permeability of the lens membranes; and the second is the Na⁺-K⁺-ATPase pump that continuously extrudes sodium ions and concentrates potassium ions. The intraocular fluids

contain a high level of the sodium ion and low potassium ion, while the cations in the lens have the opposite composition of high potassium and low sodium. Thus, if allowed to come to equilibrium sodium ion would enter and potassium ion would leave the lens (Ponder., 1961). Because the lens membranes are impermeable to proteins the situation which allows for the free exchange of cations would eventually lead to swelling. In the lens, however, the cation pump mechanism linked to active metabolism normally prevents this from happening. The ion are constantly undergoing influx and efflux the lens and the forces that move the cations are the differences in chemical potential of the lens and the active transport mechanism which is primarily located in the epithelium.

Maintaining the pump leak balance is crucial to preserving the viability of the lens. Decreased activity of $\text{Na}^+\text{-K}^+\text{-ATPase}$ pump activity results in imbalance, a net increase in electrolytes results leading to an influx of H_2O . When the gain in sodium ion can no longer be compensated by a loss in potassium ion, chloride ions enter the lens to maintain electroneutrality and it is at this point that increase in hydration begins (Kinoshita., 1974, Kinsey., 1965).

Results of present study shown that accumulation of sodium ion and loss of potassium ion in glucose 55 mM treated groups resulted in opacification of lens, revealed that ionic imbalance induced cataractogenesis. The echitamine treated group had shown that higher $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity whereas lower concentrations of sodium ion in the homogenate culture, revealed that it has significant anti cataract activity by preventing the alteration of sodium ion and potassium ion imbalance by direct effect on lens membrane $\text{Na}^+\text{-K}^+\text{-ATPase}$ (Unakar and Tsui., 1980, Chylack and Kinashita., 1969).

Whereas other alkaloids used in the study where devoid of activity of study. Incubation in the presence of high glucose (55 mM) concentration simulates a state of clinical diabetes, thus a can be a choice to retard the progression of diabetic cataracts (Chylack and Kinashita., 1969). However, higher concentrations of echitamine may show better anticataract activity, and further evaluation is required. This in vitro study may not directly correlate with the in vivo conditions. Therefore, in vivo studies in different animal models are required for further elucidation.

REFERENCES

- Barker FM. Dietary supplementation: effects on visual performance and occurrence of AMD and cataracts. *Curr Med Res Opin.* 2010 26(8):2011-23.
- Ceriello A, Russo PD, Amsted P, Cerutti P. High glucose induces antioxidant enzymes in human endothelial cells in culture. *Diabetes* 1996;45:471-7.
- Chakravarthi RN, Chakravarthi D, Sur R. A simplified method for isolation of Echitamine. *Bull Calcutta Sch Trop Med.* 1968;16(3):81-2.
- Chandorkar AG, Albal MV, Bulakh PM, Muley MP. Lens Organ Culture. *Indian. J Ophthalmol* 1981;29:151-2.
- Chylack LT, Kinashita JH. A biochemical evaluation of a cataract induced in a high glucose medium. *Invest Ophthalmol* 1969; 8:401-12
- Harding JJ, Rixon KC. Carbamylation of lens proteins: a possible factor incataractogenesis in some tropical countries. *Exp Eye Res* 1980;31:567-71.
- Kapoor LD:Handbook of Ayurvedic Medicinal Plants. Florida: CRC Press Inc; 1990:61. 72
- Kinoshita JH, Merola LU, Dikmak E. The accumulation of dulcitol and water in rabbit lens incubated with galactose. *Biochem Biophys Acta* 1962; 62:176-78
- Kinoshita JH. Cataracts in galactosemia. The Jonas Friedenwald Memorial Lecture, *Invest. Ophthalmol.* 4: 786, 1965.
- Kinoshita JH. Mechanism initiating cataract formation. *Invest Ophthalmol* 1974; 13:713-24
- Kinsey VE. 1965. The pump-leak concept of transport in the ocular lens. In: *The Structure of the Eye*, Rohen, J. W., editor. Stuttgart, , F. K. Schattauer-Verlag 383-394.
- Klein BEK, Klein R, Lee KE. Cardiovascular diseases, selected cardiovascular diseases risk factors and age related cataract: The beaver dam eye study. *Am J Ophthalmol* 1997; 123:338- 46.
- Mathew MC, Ervin AM, Tao J, Davis RM. Antioxidant vitamin supplementation for preventing and slowing the progression of age-related cataract. *Cochrane Database Syst Rev.* 2012; 13: 6
- Ponder E .1961. *The cell membrane and its properties.* The Cell, Academic Press, New York.
- Quillen DA. Common causes of vision loss in elderly patients. *Am Fam Phys* 1999;60(1):99-108
- Unakar NJ, Tsui JY. Measurement of $\text{Na}^+\text{-K}^+\text{-ATPase}$ activity. *Invest Opth Vis Sci* 1980; 19: 378-85

How to cite this article:

Lokesh J Shetty. Evaluation of Echitamine, Ditamine and Echitenine for Anti-cataract Activity using in vitro cataract Model. *J App Pharm Sci*, 2015; 5 (05): 065-067.