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Studies on the anticataract activity of echitamine, an alkaloid from the stem bark of *Alstonia scholaris*.

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ABSTRACT

The phytoconstituent echitamine isolated from stem bark of *Alstonia scholaris* was subjected for in vitro cataract prevention study on glucose induced cataract model. Goat lenses were incubated with test drugs in different concentrations at room temperature for 72 h. Biochemical parameters studied in the lens were electrolytes Na^+ , K^+ and Na^+ - K^+ -ATPase activity. Cataractous lens treated with drug shown lower Na^+ , higher K^+ and higher Na^+ - K^+ -ATPase activity. Lens treated with echitamine prevented formation and progress of cataract induced by glucose.

Key words: Cataract, Echitamine, *Alstonia Scholaris*

INTRODUCTION

Cataract is a multifactorial disease occurs mainly due to formation of large protein aggregates in the lens. The research has shown that post translational modification of lens crystallins such as oxidation, glycation, carbamylation, transamidation, phosphorylation and proteolysis lead to clouding of lens [1, 2, 3]. 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 formation of cataract [3]. The enzyme aldose reductase also plays an important role in pathogenesis. The aldose reductase acts on the sugars 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; causes hyper tonicity responsible for formation of cataract [4]. The higher glucose level also leads to the formation of superoxide radical and H_2O_2 [5]. 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 Na^+ and loss of K^+ with hydration and enlargement of the lens fibers leading to cataract formation [6].

A number of drugs were tried for anticataract activity such as aldose reductase inhibitors, NSAIDS, and a group of miscellaneous agents but none are found to be effective. Therefore the study was undertaken to find the efficacy of phytoconstituent Echitamine isolated from stem bark of *Alstonia scholaris* in the prevention of experimental cataract induced by glucose.

MATERIALS AND METHODS

Isolation of Echitamine (6)

500g of powdered bark of *Alstonia scholaris* was taken and macerated with 90% alcohol for 2 days. Then it was filtered and powder was pressed to collect the alcoholic extract. This procedure was repeated for another two times with same powder. Then all the alcoholic extract were combined. The concentrated extract kept for some days to separate non nitrogenous products. Then it was filtered and filtrate is used for isolation of Echitamine. The filtered mass was treated with 50ml of 20% acetic acid, white precipitate formed, then again filtered, clear filtrate was then concentrated to 200ml. The concentrated extract was then extracted with 50ml ether for 5 times and ether layer is separated. Then extract was treated with 50 ml chloroform for 5 times and chloroform layer is separated. The aqueous solution is filtered and the filtered extract is then added with 100ml of 20% NaOH solution. This solution is then extracted with 50ml of chloroform for 5 times, chloroform layer combined and kept for 2 days and brought down to residual volume. The residue is made acidic with 10% alcoholic HCl, the Echitamine hydrochloride crystallized out. The separated echitamine was then subjected to characterization.

Characterization of Echitamine

Brown crystalline powder of isolated compound was subjected for characterization using IR (KBR), ¹NMR (DMSO) and FAB mass. Analysis confirmed the purity of test drug confirmed the Molecular formula of test drug as [C₂₂H₂₉N₂O₄]⁺. Molecular weight and Rf factor of isolated drug were 385 and 0.8 [Toluene: ethylacetate: diethyl amine (7: 2:1)]. The colour of the spot was orange with Dragendroff's reagent. Melting Point was 204-207°C. The spectral datas of the compound as follows

IR (KBR):- 3377.71 cm⁻¹ (OH), 3262.0 cm⁻¹ (Indole N-H), 1724.05 cm⁻¹ (COOCH₃), 2858.95 cm⁻¹ (C-H), 1632.4 cm⁻¹ (C=C); ¹HNMR (DMSO):- δ 1.20 to 2.08 (2H, 2XCH), δ 1.20 to 2.08 (8H, 4X2CH₂), δ 6.12 to 7.03 (5H, m, aromatic ring + NH), δ 5.57 (1H, Q, olefinic proton), δ 4.96 (1H, S, OH), δ 3.50 (3H, S, COOCH₃), δ 2.50 (3H, S, N Me), δ 1.78 to 2.08 (3H, dd, allylic C-Me), δ 3.92 (2H, m, H-17), δ 5.05 (1H, t, OH); FAB MASS: - 386 (M+1, 48%), 271 (10%), 257 (8%), 255(5%), 218 (93%), 203 (65%), 189 (50%), 175 (38%), 159 (45%), 147 (60%), 135 (80%), 119 (100%), 95 (95%)

Chemicals

Chemicals used in present study were of analytical grade.

Preparation of lens culture

Goat eye balls were obtained from the slaughterhouse. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 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 and pH 7.8 for 72 h. Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55 mM was used to induce cataract [7].

Test drug concentration and Groups

Three concentrations of the test drug were chosen in accordance to the OECD guidelines. Concentrations were chosen in such a way that the middle dose was ten times smaller than the LD₅₀ (300mg in rats per oral) value of the test drug, high dose which was twice that of middle, and the lowest was 50% of middle concentration.

A total of 30 lenses were divided into following categories (n=6 in each group):

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

Group II: Glucose 55mM [Control - Cataractous]

Group III

A: Echitamine 15 mg/ml of lens culture + Glucose 55 mM

B: Echitamine 30 mg/ml of lens culture + Glucose 55 mM

C: Echitamine 6 mg/ml of lens culture + Glucose 55 Mm

Homogenate preparation

After 72 h of incubation, lens homogenate was prepared in Tris buffer (0.23M, pH 7.8) containing 0.25X10⁻³sub M EDTA and homogenate adjusted to 10 % w/v.

The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters.

Biochemical estimation

Electrolyte (Na^+ & K^+) estimation was carried out using flame photometry. Na^+ - K^+ -ATPase activity measured in accordance to the method of suggested by Unakar & Tsui [9].

Visual evaluation

Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh was observed through the lens as a measure of lens opacity.

Statistical analysis

All data were expressed as mean \pm 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.

Table 1: Na^+ , K^+ and Na^+ - K^+ -ATPase activity after 72 h of incubation

Study Groups	Na^+ [meq/gm]	K^+ [meq/gm]	Na^+ - K^+ -ATPase activity [$\mu\text{gP/gm}$]
Group I	52.9 \pm 12.1*	10.4 \pm 1.3***	42.9 \pm 2.1***
Group II [Control]	208.7 \pm 23.7	6.6 \pm 0.4	15.7 \pm 3.9
Group III A	171.5 \pm 24.9	7.2 \pm 1.4	24.8 \pm 9.8
Group III B	189.5 \pm 23.4	7.8 \pm 1.7	24.5 \pm 4.0
Group III C	113.0 \pm 24.4*	9.5 \pm 2.4**	31.6 \pm 3.4**

Values are mean \pm SD. n=10 for each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ as compared with their corresponding value in glucose 55 mM group.

RESULTS AND DISCUSSION

Incubation of lenses with glucose 55 Mm has shown opacification starting after 8 hrs at the periphery, on the posterior surface. This progressively increased towards the centre, with complete opacification at the end of 72 hrs of incubation. The visual evaluation has shown that the test drugs retard the progression of lens opacification, compared with Group I and II.

Group I has shown lower Na^+ ($P < 0.05$), higher K^+ ($P < 0.001$) and higher Na^+ - K^+ -ATPase activity ($P < 0.001$) compared with Group II [Table 1]. Group IIIC has shown moderately high K^+ ($P < 0.01$), moderately high Na^+ - K^+ -ATPase activity ($P < 0.01$) and moderately decrease in Na^+ concentrations, compared with Group II. [Table 1] Group IIIA and B were nonsignificant.

The study has shown that higher Na^+ - K^+ -ATPase activity whereas lower concentrations of Na^+ ions with test drug treated groups. Therefore, the test drug seems to prevent the alteration of Na^+ and K^+ imbalance, which may be due to a direct effect on lens membrane Na^+ - K^+ -ATPase [8, 9].

Incubation of lens with high glucose concentration simulates a state of clinical diabetes [9]. A preventive role of test drugs suggest in the preventing and/or retarding the progression of diabetic cataracts. However, higher concentrations of Echitamine may show better anticataract activity, and further evaluation with higher concentrations is required.

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