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Anticataract activity of *Areca catechu* and Arecoline hydrobromide through inhibition of polyol pathway for the management of diabetic complication

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Abstract

Phytotherapy has proven to be effective in the management of diabetes and diabetic complications. Diabetes has been identified as one of the major risk factors for cataract. Cataract is defined as opacification or optical dysfunction of the crystalline lens. In the present study *Areca catechu* (AC) and Arecoline hydrobromide (AHB) were evaluated as possible inhibitors of aldose reductase (AR: a key enzyme in cataractogenesis) and the plant is previously reported for its antioxidant and antidiabetic activities. Anti cataract activity of AC and AHB was demonstrated using sugar induced lens opacity model, where the chick lenses were incubated in Kreb's ringer bicarbonate buffer pH 7.5 (supplemented with Taxim and streptomycin) containing 55mM glucose (cataractogenesis) with epalrestat AC extract and AHB at a concentrations of 1µg/ml, 100µg/ml and 10µg/ml respectively for 24 hrs at 37°C with 5% CO_2 and 95% air. After 24 hrs polyol (galactitol) levels in incubated lenses were measured spectrophotometrically. When compared to the standard drug epalrestat, the plant extract and its constituent demonstrated significant AR inhibitory activity. Cataract induced lenses had a higher galactitol content. However, lenses treated with epalrestat, AC and AHB contained lower content of galactitol. The current study found that AC and AHB prevented the formation and progression of cataract by glucose, as evidenced by lens transparencies with photographic evaluation and lens galactitol level.

Keywords : Aldose reductase, Anticataract, Polyol, *Areca catechu*, Arecoline hydrobromide.

1.Introduction

Cataract is a visual impairment caused by opacification or optical malfunction of the crystalline lens. It lowers the amount of light that enters the eye, causing eyesight to deteriorate. Apart from senile cataract, other variables that enhance the incidence of cataract include oxidative stress, diabetes, excessive ionising radiation exposure and inflammatory eye illnesses. Cataractous lenses can be surgically replaced with artificial lenses, but the problem persists epidemiologically due to the cost and post-operative consequences of surgery [1].

Diabetic people have a higher rate of cataract occurrence and development. Extracellular glucose diffuses into the lens uncontrolled by the hormone insulin during hyperglycemia, making the lens one of the body components most affected by diabetes mellitus. The proteins in the lens have a very long half-life and there is almost no protein turnover, which can lead to post translational modification [2]. The synthesis and accumulation of excessive sorbitol (polyols) in the lens fibres, as well as the resulting osmotic stress, are the main causes of cataract formation [3]. Sorbitol is produced by aldose reductase using NADPH and does not easily cross cell membrane, it can build up in cells and cause damage by disrupting osmotic homeostasis [4].

AR inhibition has been identified as a potential alternative target [5] and clinical evidence suggests that using natural therapies particularly plants or plant derived pure chemicals with aldose reductase inhibitory effects, can help prevent and slow down diabetic complications [6].

Areca palms are primarily grown in south and southeast Asian countries like India, China, Bangladesh, Indonesia, Myanmar, Thailand, Malaysia, Vietnam and Philippines, Arecanut, betel nut or supari is the name given to its fruit or seed. Polyphenols, primarily flavonoids and tannins, polysaccharides, proteins, fats, fibres and alkaloids are the major constituents of arecanut. Arecoline is the most active and physiologically active of the alkaloids found in arecanut and it has stimulating effect [7].

Arecoline, an alkaloid found in betel nut, is said to have hypoglycemic properties [9]. In this context, the current study was designed to investigate the aldose reductase inhibitory activity and *in vitro* anticataract activity *Areca catechu* and its constituent Arecoline.

2.Material and Methods

2.1 Materials

DL- Glyceraldehyde, NADPH, Kreb's ringer buffer medium and Dulcitol were obtained from Hi media laboratories (Mumbai, India). *Areca catechu* seed extract was kindly gifted by Amsar labs, Indore. Arecoline hydrobromide was purchased from Avra labs. Epalrestat was kindly donated by Symed labs (Hyderab, India). All other chemicals were of analytical grade.

2.2 Animals

Male wistar rats (150 gm) were obtained from Vyas labs (Hyderabd, India) and housed at 25℃ with relative humidity 45-55% under a natural light: dark cycle with unrestricted access to food and water. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), 13/IAEC/UCPSC/KU/2022.

2.3 AR inhibitory activity *in vitro*

2.3.1 Tissue homogenates preparation

Eyes and kidneys of normal wistar rats were removed immediately after sacrifice. The posterior technique was used to enucleate the eye lenses. Both the lens and kidneys were

homogenised separately in three litres of 0.1M sodium phosphate buffer (pH 6.2) and centrifuged at 16,000 rpm for 30 minutes at 4℃ and the supernatant thus obtained was used as crude AR preparation [10]. The enzyme preparation was assessed for the protein content, enzyme activity and specific activity by using previously described methods [11,12].

2.3.2 AR inhibitory assay

The AR inhibitory activity experiment was carried out as described before by Jung *et al.*, [13]. Briefly AC extract at concentrations of 10, 50 and 100µg/ml, AHB at concentrations 1,5,10 µg/ml and epalrestat at concentrations of 0.1,0.5,1 µg/ml were prepared individually in 10% DMSO. The reaction mixture included 300µl of 0.15 mM NADPH, 300µl of test or standard drug solution (10% DMSO in blank), 300µl of crude enzyme preparation and sodium phosphate buffer to make the final volume up to 2.7 ml. the reaction was started by adding 300µl 10mM DL-Glyceraldehyde as a substrate (double distilled water as a blank) and absorbance was measured at 340 nm for 1 minute at 5 secons intervals using a double beam UV spectrophotometer (SL 210, Elico, India). In triplicates, the absorbance was measured for all the concentrations. Each sample's AR inhibitory activity was determined using the following formula:

% inhibition = $[1- \frac{\Delta A\ Sample - \Delta A\ Blank}{\Delta Acontrol - \Delta ABlank}] *100$

Where, ΔA sample is a decrease of absorbance per minute with test or standard. ΔA blank is decrease in absorbance of blank solution, while ΔA control is with 10% DMSO in place of sample solution.

3. *In vitro* anti cataract activity

3.1 Preparation of lens culture

Fresh chick eye balls were collected from the slaughter house and lens were isolated by extracapsular extraction and washed with saline. The experiment was conducted on the same day. All the lens were incubated in Kreb's ringer bicarbonate buffer supplemented with Taxim and Streptomycin to prevent the bacterial contamination for 24hrs at 37℃ CO_2 and 95% of air. Cataract was induced in lenses by glucose at a concentration of 55mM [14]. Lenses were divided into the following groups (n=3):

Group I (Negative control) : Kreb's-Ringer bicarbonate buffer with 10mM glucose.

Group II (Positive control) : Kreb's-Ringer bicarbonate buffer with 55mM glucose.

Group III (AC) : Kreb's-Ringer bicarbonate buffer with 55mM glucose and AC 100µg/ml.

Group IV (AHB) : Kreb's-Ringer bicarbonate buffer with 55mM glucose and AHB 10 µg/ml.

Group V Epalrestat (EPST) : Kreb's-Ringer bicarbonate buffer with 55mM glucose and EPST 1µg/ml.

After incubation, the transparency of lens was observed by placing the different group of lenses on a wired mesh with posterior surface touching the mesh. The pattern of mesh number of squares clearly visible through the lens was observed to measure the lens opacity. The degree of opacity was graded as follows: [15]

1. Absence of opacity : 0
2. Slight degree of opacity : +
3. Presence of diffuse opacity : ++
4. Presence of extensive thick opacity :+++

3.2 Estimation of Polyol concentrations

The method described by Halder et al., [16] was used to estimate the lens polyols. After homogenising the lenses in 0.6 N perchloric acid, they were centrifuged for 30 minutes at 5000rpm. After centrifugation, the supernatant was collected and neutralised with 2N KOH.

The supernatant was separated and then centrifuged again after being neutralised with 2N KOH. The obtained supernatant was reacted for 30 minutes on a water bath with 0.2 ml of 0.03 M periodic acid , 0.2 ml stannous chloride (freshly prepared) and 2 ml of Chromotropic acid (0.2%) to produce a purple coloured complex. Using a double beam UV spectrophotometer, the absorbance was determined at 570 nm (SL 210, ELico, India). A galactitol parallel standard was used to determine the concentrations.

4.Results

Table 1 shows the in vitro AR inhibitory activity of the plant extract and its constituents on rat lens and kidneys. The enzyme protein concentration, enzyme activity and specific activity of rat lens aldose reductase (RLAR) in the lens homogenate were found to be 1.9 mg/ml, 13.26 U/ml and 6.97 U/mg, respectively. Similarly, the crude enzyme protein concentration, enzyme activity and specific activity of the kidney homogenate of rat kidney aldose reductase (RKAR) were found to be 2.3 mg/ml, 12.18 U/ml and 5.29 U/mg, respectively. The plant extract inhibited RLAR and RKAR in a concentration – dependent manner with the highest activity obtained at the highest concentration (100 µg/ml) with IC_{50} values of 32.91 µg/ml and 27.83 µg/ml, respectively. The IC_{50} values of AHB against RLAR and RKAR were found to be 1.66 µg/ml (7.03µM) and 1.95 µg/ml (8.28µM) respectively, while the IC_{50} values of standard epalrestat were 0.49 µg/ml (1.55 µM) and 0.52 µg/ml (1.62µM) for RLAR and RKAR, respectively.

Table 1: Effect of *Areca catechu* and Arecoline on RLAR and RKAR reductase inhibitory activity *in vitro*.

Sample	Concentration (µg/ml)	RLAR			RKAR		
		Percentage Inhibition	IC_{50} (µg/ml)	IC_{50} (µM)	Percentage Inhibition	IC_{50} (µg/ml)	IC_{50} (µM)
AC	10	42.89±1.50	32.91		43.63±0.98	27.83	
	50	52.97±0.80			57.59±1.84		
	100	79.09±0.48			78.51±0.74		
AHB	1	46.29 ± 1.11	1.66	7.03	47.37±0.74	1.95	8.28
	5	61.68 ±0.488			59.62±0.73		
	10	81.32±0.660			80.37±0.37		
Epalrestat	0.1	16.67±0.800	0.49	1.55	12.33±0.74	0.52	1.62
	0.5	58.92±1.46			57.91±0.48		
	1.0	82.49±0.64			81.75±0.64		

All values are expressed as mean ± SD, n=3. AC: *Areca catechu*; AHB: Arecoline hydrobromide; SD: standard deviation; IC_{50}: Half-maximal inhibitory concentration.

4.2 *In vitro* anti cataract activity

Photographic evaluation of chick lens transparency:

Photographic assessment of the lens shown that all the lenses incubated in the presence of kreb's ringer bicarbonate buffer with high glucose (55mM) led to the decrease in lens transparency, while the lenses incubated in the kreb's ringer bicarbonate buffer with 10mM

glucose remained transparent. Treatment with AC, AHB and epalrestat slowed the development of lens opacification, which was demonstrated by the ability to clearly see gridlines through the lens.

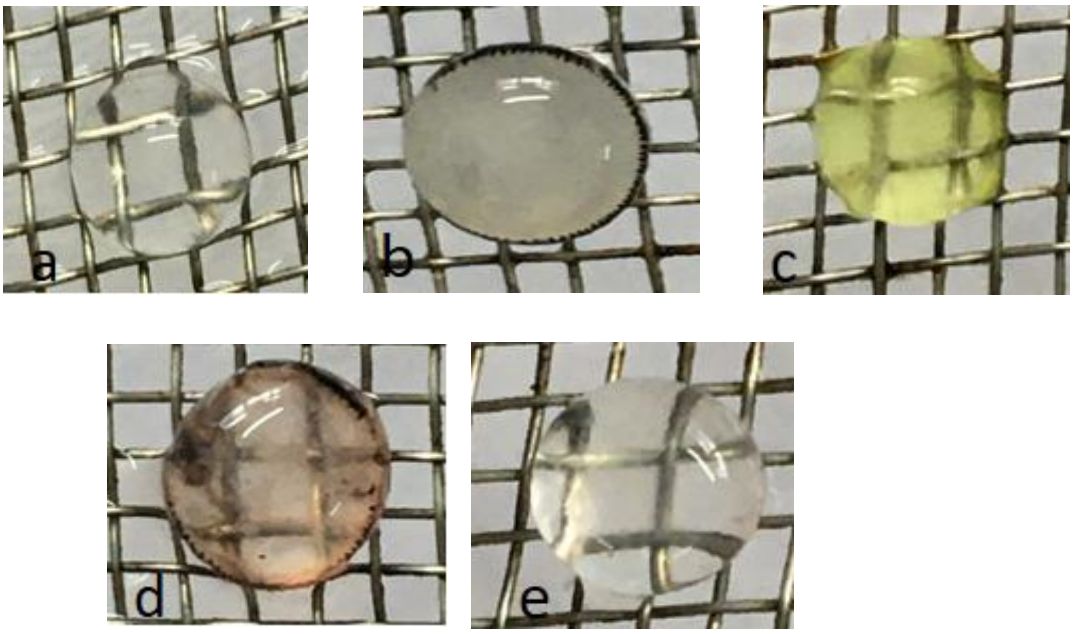

Figure 1: Effect of plant extract and its phytoconstituent on lens transparencies in sugar induced opacity model in chick lens. (a). Negative control, (b). Positive control, (c). epalrestat (1µg/ml), (d). *Areca catechu* (100µg/ml) and Arecoline hydrobromide (10µg/ml).

Table 2: This table shows the photographic evaluation of chick lens with negative control, positive control, epalrestat (standard), *Areca catechu* (AC) and Arecoline hydrobromide (AHB).

S.No	Degree Of opacity	Negative Control		Positive Control		Standard		AC		AHB	
	Hours	1	24	1	24	1	24	1	24	1	24
1	Absence Of opacity	0	0	0	0	0	0	0	0	0	0
2	Slight degree of opacity	0	0	+	+	0	+	0	+	0	+
3	Presence of diffuse opacity	0	0	++	++	0	0	0	0	0	0
4	Presence of extensive thick opacity	0	0	+++	+++	0	0	0	0	0	0

4.3 Effect of Polyol concentrations

Figure 2 depicts the effect of changes in polyol levels on the lens. Polyol levels in lenses incubated in high glucose medium were significantly higher than in negative control. The presence of AC, AHB and epalrestat in a high glucose medium significantly reduces osmotic stress caused by an increase in polyol levels, indicating a protective effect against cataract formation.

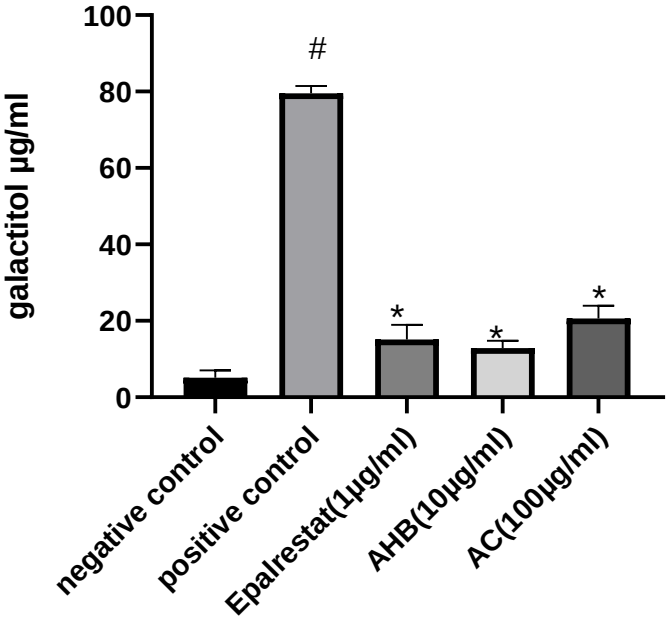

Figure 2: Effect of *Areca catechu* and Arecoline hydrobromide on polyol levels in chick lens in sugar induced opacity model. Data was analysed by one-way analysis of variance followed by Dunnett's test (n=3); # P<0.001, as compared to negative control, * P<0.001 as compared to positive control.

5.Discussion

According to the previous reports, increased expression of aldose reductase has been linked to the development of diabetic complications such as retinopathy, neuropathy, nephropathy and cataracts. Despite the fact that a number of synthetic aldose reductase inhibitors are proposed for the treatment and prevention of diabetic complications, but their use is limited due to detrimental side effects. Therefore, plants and compounds derived from plants can be used in the management and avoidance of diabetic complications.

Thus, in the present study AC and AHB were evaluated for the AR inhibitory activity. The plant was selected based on the previously reported antidiabetic and antioxidant activities [17].

Table 2 shows that the plant extract and active constituent had potent AR inhibitory activity comparable to the standard drug epalrestat. As a result, the findings of this study prompted further investigation into its effect on cataract formation *in vitro*.

The majority of studies have shown that diabetes duration and hyperglycemia pose significant risks for the development of cataracts, which is a multifunctional process [18]. The pathogenesis of cataract development is thought to involve changes in the polyol pathway, nonenzymatic glycation, and oxidative stress pathways [19].

The key event in the glucose – induced cataract is the activation of the polyol pathway with the conversion of glucose into sorbitol by AR. Because the lens cellular membranes are

sorbitol impermeable, sorbitol builds up in the lens, causing hyperosmotic cell swelling, which results in light scattering and decreased lens transparency [20].

Osmotic stress was increased in the crystalline lens as a result of hyperglycemia- induced aldose reductase mediated rise in polyol levels [21]. Furthermore, it has been demonstrated that high glucose (55mM) increases in the formation of reactive oxygen species, causing severe oxidative stress. Changes in antioxidant enzymes levels have been linked to an increase in reactive radicals, which can have negative consequences like a loss of cell membrane integrity and function [22].

Diabetic cataractogenesis in experimental animals may be caused by an increase in polyol production from reducing sugars by the AR enzyme, a key rate-limiting enzyme that aids in the development of cataracts in diabetic patients. It transforms glucose into sorbitol and galactose to galactitol [23]. AR inhibitors could be an excellent strategy for postponing or preventing cataract. Because oxidative stress is a common underlying mechanism of cataractogenesis, antioxidant defences have been shown to prevent or delay cataract [24]. Earlier research on animal models revealed a link between AR inhibitors and diabetic cataract prevention [25].

A well- known paradigm for hyperglycemia-induced cataract is *in vitro* lens organ culture [26]. In the current investigation, AC and AHB were assessed for their ARI activity-mediated anti cataract potential against high glucose-induced opacity and metabolic alterations in chick lenses in organ culture. In consistent with the above theory, lenses that were incubated in high glucose medium showed opacity when compared to lenses that were incubated in low glucose medium. The presence of AC, AHB and epalrestat in the culture medium prevented opacity induction, which can be attributed to the inhibition of lens aldose reductase by the compounds. This is supported by the fact that the presence of AC, AHB and epalrestat in the high glucose medium significantly reduced polyol accumulation.

6.Conclusion

In the current research AC and AHB were assessed for their ability to inhibit AR and in vitro anti cataract activity. According to the best of our knowledge after reviewing the scientific literature, this is the first report on AR inhibition and *in vitro* anti cataract activity. In conclusion, the findings of the current study revealed that AC and AHB showed AR inhibition and prevented accumulation of polyols. However further research is needed to assess the benefit of plant and its constituent to lower the risk of diabetic complications.

7. References

1. Lakshmi SVVNSM., Sivamallikarjuna AR., Ganapaty S., Ganga Rao B., Ramesh A., Anticataract potential of *Boerhavia diffusa* roots on galactose induced cataractogenesis, *Indian J. Exp. Biol*., 2017, 55, 838-844.

2. Kyselov Z., Stefek M., Bauer V., Pharmacological prevention of diabetic cataract, *J Diabetes complications*., 2004, 18(2), 129-140.

3. Andreas P., Ursula SE., Diabetic cataract-pathogenesis, epidemiology and treatment , *Journal of Opthalmology*., 2010, 10, 1-9.

4. Mihir Y P., Tribhuvan S., Dinesh B., Sachin K S., Dinesh P., Evaluation of anticataract activity of methanolic extract of *Trianthema decandra* leaves against galactose induced cataractogenesis, *JOJ Opthalmology*., 2019, 7(3), 001-006.

5. Baynes J W., Role of oxidative stress in the development of complications in diabetes , *Diabetes*.,1991, 40, 405-412.

6. Lim S S., Jung S H., Synthesis of flavonoids and their effects on aldose reductase and sorbitol accumulation in streptozotocin induced diabetic rat tissues, *J.Pharm. Pharmacol*., 2001, 53, 653-668.
7. Bhat S K., Ashwin D., Mythri S and Sukesh B, Arecanut (*Areca catechu* L) decreases alzheimer's disease symptoms: compilation of research works, *J. Med. Plants Stud*.,2017, 5(5), 04-09.
8. Bhat S K., Mythri S and Ashwin D., Antilipidemic activity of arecanut, *Areca catechu* L: A valuable herbal medicine, *Int. J.Herb.Med*., 2017, 5(1), 35-38.
9. Chempakam B, Hypoglycemic activity of arecoline in betel nut *Areca catechu* L, *Indian J Exp.Biol*., 1993, 31,474-475.
10. Cerelli M J., Curtis D L., Dunn J P., Nelson P H., Peak T M and Waterbury L D., Anti inflammatory and aldose reductase inhibitory activity of some tricyclic arylacetic acids, *J. Med.Chem*., 1986, 29(11), 2347-51.
11. Lowry O H., Rosebrough N J., Farr A L and Randall R j., Protein measurement with the Folin phenol reagent, *J Biol Chem*., 1951,193(1), 265-75.
12. Hayman S., Kinoshita J H., Isolation and properties of lens aldose reductase, *J Biol Chem*., 1965, 240, 877-82.
13. Jung H A., Jung Y J., Yoon N Y., Jeong D M., Bae H J., Kim D W., et al., Inhibitory effects of *Nelumbo nucifera* leaves on rat lens aldose reductase, advanced glycation end products formation and oxidative stress, *Food Chem.Toxicol*., 2008,46(12), 3818-26.
14. Sujatha D., Rao A R and veeresham C., Anticataract activity of forskolin by inhibiting polyol pathway for the prevention of diabetic complications, *Pharmacognosy Res*., 2019, 11,352-5.
15. Anjali V., Somasekhar., Sindhu G., Girish C., Peera K and Thyagaraju K., Evaluation of anticataract activity of *Syzygium cumini* seeds, *Int. J. Curr. Adv. Res.*, 2018, 7(3), 11276-11285.
16. Halder N., Joshi S and Gupta S K., Lens aldose reductase inhibiting potential of some indigenous plants, *J. Ethnopharmacol*., 2003, 86(1), 113-6.
17. Patil R., Sachin U R., Dhabale P N., Burade K B., Pharmacological activities of *Areca catechu* Linn. – A Review, *J. Pharm. Res*., 2009, 2(4), 683-687.
18. Crabbe M J., Aldose reductase inhibitors and cataract, *International Opthalmology*., 1991,15, 25-36.
19. Obrosova I G., Chung S S and Kador P F., Diabetic cataracts: Mechanisms and management, *Diabetes Metab Res Rev.*, 2010, 26,172-80.
20. Kinoshita J H., Cataracts in galactosemia investigate, *Opthalmology* (St. Louis USA).,1965, 4, 786-99.
21. Collier A and Small M.,The role of the polyol pathway in diabetes mellitus, *Br. J. Hosp. Med*., 1991,45,38-40.
22. Chatzopoulou M., Alexiou P., Kotsampasakou E and Demopoulos V J., Novel aldose reductase inhibitors: A patent survey (2006- present), *Expert opin.Ther.Pat*., 2012, 22,1303-23.
23. Kim J., Kim C S., Lee Y M., Sohn E., Jo K., Shin S D, et al., Scopoletin inhibitors rat aldose reductase activity and cataractogenesis in galactose-fed rats, *Evid Based Complement Alternat Med*., 2013.

24. Kalekar S A., Munshi R P and Kulkarni D K., Evaluation of anti-cataract potential of *Tinospora cordifolia* and *Phyllanthus emblica* in an *in vitro* sugar induced cataractic lens organ culture model, *Int.J. Pharma and Bio Sci*., 2014,5,120-30.
25. Kawakubo K., Mori A., Sakamoto K., Nakahara T and Ishii K., GP-1447, an inhibitor of aldose reductase, prevents the progression of diabetic cataract in rats, *Biol. Pharm. Bull*., 2012, 35,866-72.
26. Chandorkar A G., Albal M V., Bulakh P M and Muley M P., Lens organ culture. Methodology and preliminary observations on viability and maintenance of transparency, *Indian J. Opthalmol.*, 1981,29, 151-2.