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Original Research Article

Anticataract activity of pioglitazone by using in-Vitro goat lens model

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ABSTRACT

In the present study we have selected antioxidants agents like Pioglitazone and Ascorbic acid were subjected for anti-cataract activity by in vitro glucose induced cataract model. In the procedure, goat lenses was incubated along with the aqueous humor solution containing 55mM glucose with Enalapril as a standard compound and Pioglitazone with varied concentration for the time interval of 72 hours at room temperature. There was a formation of blur layer on the goat eyeball occurs after 10-12 hours and this process complete after 72 hours. The cataract inducing lenses showing higher level of Na⁺, MDA (P<0.001) along with the decreases in sodium-potassium ATPase activity and water-soluble protein content. The goat lenses treated with Ascorbic acid 40 µg/ml and Pioglitazone in concentrations of 15, 30, and 60 µg/ml showed increased protein content and prevent the formation of cataract.

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1. Introduction

The disease cataract is the opacification of lenses frequently occurs in diabetic patient with old age because of increased levels of glycosylated haemoglobin are involved in increased risk of cataract formation.¹ Many cataract inducing factors have been reported according to the various experimental data; the biochemical reason of cataractogenesis is still not clear till date. The cataract occurs because of multiple factors, mainly happen due to the protein aggregation on the eye lenses. The lenses Na⁺ — K⁺ -ATPase activity shows very important action in maintenance of transparent nature of the lens, and its imbalance leads to deposition of Na⁺ and loss of K⁺ with water absorption of the lens fibres leading to cataract formation.² Numbers of drugs have been already used for treatment of cataract but not a single from them proved useful treatment of cataract.³ The aldose reductase is

enzyme probably plays a role in the development of this eye problem like cataract.⁴ The aldose reductase show its action on sugar molecules like glucose, galactose and xylose and convert them into their respective alcohols by biochemical pathways these alcohols are known as polyols accumulate within the lens there by producing osmotic effects. Hence polyols does not have a capacity for diffusing out easily nor metabolizes rapidly and causes hyper tonicity responsible for formation of cataract.⁵ The oxidative mechanism also plays a crucial role in biological phenomena including cataract formation. The formation of superoxide radicals in the aqueous humour and in lens, lens and its derivatization to other potent oxidants may be responsible for initiating various biochemical toxic reactions leading to formation of cataract.⁶ Angiotensin converting enzyme inhibitors have been shows protection from free radical damage in many experimental procedures.

Ascorbic acid (ACE Inhibitor) was shown potent anticataract activity in vitro due to antioxidant and free radical scavenging activity.^{7,8} Hence we take Ascorbic acid

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as standard and measure various parameters including (Na^+ & K^+) estimation, Na^+ - K^+ ATPase activity, Proteins (total proteins and water soluble proteins) and malondialdehyde (MDA) in vitro on goat lenses.

2. Materials and Methods

2.1. Drugs

Drugs Ascorbic acid, Penicillin and streptomycin were obtained from Loba chemicals, Spectrochem Pvt. Ltd. and some local chemical supplier.

2.2. Experimental procedure

2.2.1. Collection of eyeballs

Goat eyeballs used for the study were collected from the local slaughterhouse and stored at $0-4^{\circ}\text{C}$.

2.2.2. Lens culture

Fresh goat eyeballs used for the study are withdrawal from slaughterhouse. Artificial aqueous humour is used for anticataract activity. Aqueous humour contains (NaCl 140 mM, KCl 5mM, MgCl_2 2 mM, NaHCO_3 0.5 mM, NaHPO_4 0.5 mM, CaCl_2 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.4 by addition of NaHCO_3). Penicillin G and streptomycin 250 mg added for prevention of bacterial growth.

2.2.3. Cataract formation

The glucose solution having concentration 55mM was used for the cataract formation. Higher concentration of glucose metabolizes by the sorbitol pathway. Cataract was formed due to the accumulation of polyol (Sugar + Alcohol) which causes oxidative stress and over-hydration which forms cataract. All these lenses were incubated in aqueous humour which is artificially prepared with different concentration of Glucose for 72 hours.

2.2.4. Study design and groups

Goat lenses were divided into six groups of six lenses each following Table 1

2.3. Homogenate preparation

The homogenate is prepared by 72 hours incubation, homogenate of lenses was prepared by using Tris buffer (0.23 M, pH-7.8) containing 0.25×10^{-3} sub M EDTA and homogenate makeup up to 10 % w/v. The prepared homogenate was centrifuged at 9,000 G at 4°C for 1 hour and the supernatant for the final solution was isolated from the centrifuge tube which is used for estimation of biochemical parameters. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH-7.4).

Table 1: Treatment groups for anticataract activity

Group no	Group name	Treatment	Drug dose
I.	Normal Control	Aqueous Humor + 5.5 mM Glucose	-
II.	Negative Control	Aqueous Humor + 55 mM Glucose	-
III.	Standard	Aqueous Humor +55 mM Glucose + Standard (Ascorbic Acid)	40 $\mu\text{g/ml}$
IV.	Test 1	Aqueous Humor + 55 mM Glucose +Pioglitazone	15 $\mu\text{g/ml}$
V.	Test 2	Aqueous Humor + 55mM Glucose +Pioglitazone	30 $\mu\text{g/ml}$
VI.	Test3	Aqueous Humor + 55mM Glucose + Pioglitazone	60 $\mu\text{g/ml}$

2.4. Biochemical analysis

The electrolyte Sodium and Potassium (Na^+ and K^+) was estimated by using flame photometry. The sodium Potassium ATPase activity was performed by using Unakar and Tsui method⁹ and estimation of protein was done by Lowry's method.¹⁰The oxidative stress level was analysed by Wilbur's method.¹¹

2.5. Statistical analysis

The all experimental data was shown as mean \pm SEM. The whole data was evaluated by analysis of variance (ANOVA) and then post hoc- Dunnett's test using Graph Pad Prism software, version 4.02.

3. Results and Discussion

3.1. Photographic evaluation

After all experimental procedure lenses were placed on graph paper with the posterior surface touching the graph and observed by using magnifying lens. All the 5.5 mM Glucose (Normal lens), 55 mM Glucose (Cataract induced lens), 40 $\mu\text{g/ml}$ Ascorbic acid (Standard) were analysed by placing on graph paper. All other three lenses treated with Pioglitazone with concentrations of 15, 30 and 60 $\mu\text{g/ml}$ respectively as shown inFigure 1.

Pioglitazone was subjected for in vitro anticataract activity by goat eye lens model. Pioglitazone has multiple biological functions here which play anticataract activity.

Pioglitazone significantly protected the lens morphology and activity and clarity: 50% of the eyes had almost clear lenses; in contrast, 100% of the negative control eyes developed dense nuclear opacity. From the current study, it is evident that Pioglitazone protects the lens

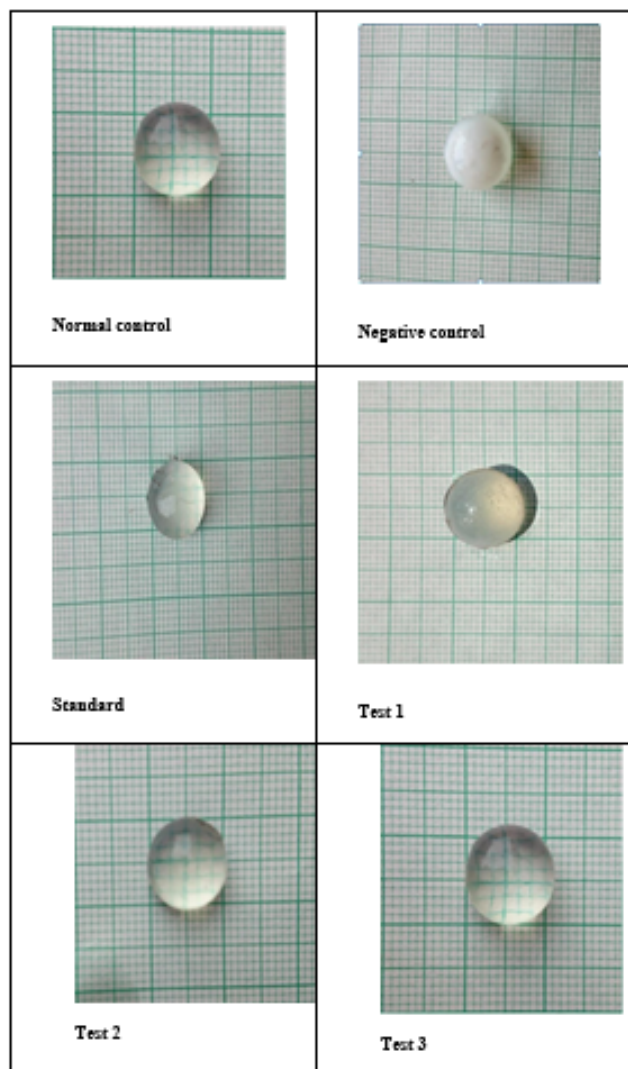


Fig. 1: Anticataract activity results

Table 2: Effect of pioglitazone on degree of opacity on lens by glucose-Induced cataract

Sr. No.	Compound	Degree of opacity
1	Normal	0
2	Negative control (Glucose 55 mM)	3
3	Positive control (Ascorbic acid 40µg/ml)	1
4	Test 1 (Pioglitazone 15µg/ml)	2
5	Test 2 (Pioglitazone 30µg/ml)	1
6	Test 3 (Pioglitazone 60µg/ml)	0

Normal control	0 degree opacity occurred, clear lens is obtained.
Negative control	The presence of extensive thick opacity, because of higher concentration of glucose- induced cataractogenesis.
Positive control (Ascorbic acid 40µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test 1 (Pioglitazone 15µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test 2 (Pioglitazone 30µg/ml)	Lenses show the slight degree of opacity, clear lens was not found.
Test 3 (Pioglitazone 60µg/ml)	Zero degree opacity is occurred, the clear lens is obtained. Test drug inhibits cataractogenesis.

against oxidative stress. These results in glucose-induced cataracts in vitro studies not only demonstrate the protective effect of Pioglitazone but also indicate that it prevents cataractogenesis by virtue of its antioxidant properties. Pioglitazone, therefore, may be useful for prophylaxis or therapy against cataract. After 72 hr. of incubation in glucose 55 mM, the lens becomes completely opaque as against lenses in normal control. Incubation of lenses with Pioglitazone and ascorbic acid both the concentrations were used, which seem to retard the progression of opacification compared with lenses incubated in glucose 55 mM (Negative Control). The effect of Pioglitazone on the positive control groups, showed considerable retardation in the progression of lens opacification and which is near normal when compared to negative control.

N=6, values are expressed as Mean ± SEM. Comparison were made as following, # $p < 0.05$, ## $p < 0.01$ when compared with normal control. * $p < 0.05$, ** $p < 0.01$ when compared with negative control. (Values are compared on 72hr by one way ANOVA Dennett test) N. S. — non-significant.

The 55 mM Glucose treated lenses (Group-II) showed significantly low concentrations of proteins (total and water-soluble proteins) in the lens homogenate ($P < 0.01$) compared with normal lenses (Group-I). Ascorbic acid treated lenses (Group-III) and Lenses treated with Pioglitazone (Group-IV, V, VI) showed higher concentrations of proteins (total and water-soluble proteins) ($P < 0.01$) compared with 55 mM Glucose treated lenses (Group-II).

4. Conclusion

In the present work, Pioglitazone treated group shows the increase in protein content (water-soluble) by prevention of cataractogenesis. This cataract is due to the higher glucose concentration. Pioglitazone shows anticataract activity due to presence of antioxidant activity and prevention of the cataract forming factors.

Table 3: Effect of pioglitazone on protein levels (total proteins and water-soluble proteins) in goat lens homogenate after 72 hours of incubation in glucose 55 mM induced cataract

Group No.	Treatment	Total proteins [mg/gm]	Water-Soluble proteins [mg/gm]
1	Normal lens Control (5.5 mM Glucose)	201.49± 2.239	81.664± 3.044
2	55 mM Glucose	169.24±2.095##	60.445± 3.056##
3	55 mM Glucose + Ascorbic acid 40µg/ml	209.33 2.699**	74.514± 2.146
4	55 mM Glucose 55 mM + Pioglitazone 15µg/ml	181.20± 2.139	71.864± 1.278*
5	55 mM Glucose + Pioglitazone 30µg/ml	207.34± 1.648**	73.844± 1.645**
6	55 mM Glucose + Pioglitazone 60µg/ml	212.70± 1.686**	75.446± 1.147**

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6. Source of Funding

None.

7. Conflict of Interest

None.

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