CORRELATING ANTIDENATURATION AND ANTICATARACT: A NOVEL APPROACH OF TREATING CATARACT

Author :V.V.Nimbalkar[1] ,A.S.Singar[2] ,K.D.Joshi[3]

[1] Assistant Professor of Pharmacology

Dr V.V.P.F'S College of Pharmacy , Ahmednagar

[2]Student final year B. pharmacy

[3]Student final year B. pharmacy

[1]Department of Pharmacology, Dr. V.V.P.F's College of Pharmacy ,Vilad Ghat, Post :MIDC,Ahmednagar,Maharashtra,India.414111.

ABSTRACT

Objectives: To evaluate in vitro antioxidant and anticataract activity of Diosgenin against glucose induced cataractogenesisusing goat lenses.

Materials and Methods:Transparent isolated goat lenses were incubated in artificial aqueous humor and divided into five experimental groups. The Diosgenin at a dose of 20µg/ml is incubated simultaneously with glucose(55mM)and glucose (5.5mM) for a period of 72 hours ,Ascorbic acid (20µg/ml)is used as the standard drug.At the end of incubation lense opacity is measured by photographic evaluation. Antidenaturing activity is evaluated by taking different concentration of Diosgenin (10p.p.m-500p.p.m)with egg albumin and subjected for protein denaturation by using heat for 15 min. Standard drug used is Diclofenac sodium,at the end protein denaturation is analysed by taking absorbance at 440nm and 660nm.

Results:Diosgenin exhibited concentration dependent inhibition of thermally induced protein denaturation and also shows significant inhibition of cataractogenesis of eye lenses by Diosgenin at conc. 20 p.p.m.

Conclusion:The present study supports Diosgenin as an antidenaturing and anticataract agent.

Keywords: Cataract, Ascorbic Acid ,Diosgenin,Antioxidants,Antidenaturation

INTRODUCTION

Cataract is the opacification or optical dysfunction of the crystalline lens,associated with the breakdown of the eye lens micro-architecture,which interferes with transmission of light onto the retina. Several biochemical processes such as oxidative stress, altered epithelial metabolism,calcium accumulation,calpin-induced proteolysis, crystalline, phase transition and cytoskeletal loss occur during the development of cataract[1].Cataract can be classified into four types;nuclear,cortical,posterior subcapsular(PSC),and mixed. Although the etiology of each cataract type remains elusive,cataracts are known from studies on many animal models and humans to be associated with damage or death of lens epithelial cells(LECs)[2]. Cataract is the major cause of blindness worldwide and covers around 45%of overall visual impairment . Cataract

is mainly responsible for almost 80% blindness in India[3]. Cataract is also produces by the advanced glycation end products(AGE)is cause of blindness world-wide[4].

Surgery is the only effective treatment for cataract and the exact mechanism is not clear. Although the surgery is recognized as being one of the safest procedure, there are significant rate of complications,leading to irreversibly blind eyes.Pharmacological interventions to delay or inhibit lens opacification is yet at experimental stage. Studies are being conducted to explore the mechanism of cataractogensis using Various models of cataract and to target crucial steps to stop this process .Limitations in accessibility and affordability of cataract surgical services make it more relevant and important to look into alternative pharamacological measures for treatment of this disorder. Thus much eagerness is being laid on identification of natural compounds that will help to prevent cataractogenesis[5].

Under physiological conditions,glucose is metabolized through the glycolytic pathway. An excess amount of glucose is converted to sorbitol by enzyme aldose reductase via polyolpathway. The glucose converted into sorbitol by utilizing NADPH results in the reduction of NADPH/NADP+. Sorbitol does not easily cross cell membrane. Intra lenticular accumulation of sorbitol,leads to lens damage. As,the lens starts to swell in response to the hyper osmotic effects of polyol,membrane permeability changes resulting in an increase in lenticular sodium and decrese in the levels of lenticular potassium,reduced glutathione(GSH)[6].

Oxidative stress may also be implicated in the cataract induced by glucose and age related process due to the formation of superoxide(O_2) radicals and H_2O_2 because of these free radicals are readily react with the biomolecules[7]. The toxic effects of the reactive oxygen species are neutralized in the lens by antioxidants such as ascorbic acid,vitamin E, the glutathione system(GSH peroxidase, GSH reductase),superoxide dismutase and catalase. The enzymatic (superoxide dismutase,glutathione peroxidase, catalase) and non-enzymatic (ascorbate,glutathione,cysteine) antioxidant system activities are decreased in the lens and aqueous humor during aging and in the development of cataract[8].

Diosgenin, a steroidal sapogenin, occurs abundantly in plants such as *Dioscorea alata, smilax china,* and *trigonella foenum graecum*. This bioactive phytochemical not only is used as an important starting material for the preparation of several steroidal drugs in the pharmaceutical industry,but has revealed also high potential and interest in the treatment of various types of disorders such as cancer,hypercholesterolemia,inflammation,and several types of infections.

Therefore,this study was conducted to evaluate the efficacy of Diosgenin for its in vitro antidenaturing and anticataract activities against glucose-inducedcataractogenesis using goat lenses.

MATERIALS AND METHODS

- **Drugs and chemicals :**

Diosgeninwas obtained from Yaaro Pharmaceuticals. All other chemicals used in the study i.e. Diclofenac Sodium, Ascorbic Acid, Sodium chloride, Potassium Chloride, Magnesium Chloride, Sodium bicarbonate, Calcium chloride, Sodium phosphate, phosphate buffer, Glucose, Penicillin, Streptomycin were obtained commercially and were of analytical grade. Fresh goat lenses were obtained from slaughterhouse.

- **Inhibition of protein denaturation method[9]:**

The following procedure was followed for evaluating the percentage of inhibition of protein denaturation :-

Control Solution (50ml):

2 ml of Egg albumin (from fresh Hen's egg), 28 ml of phosphate buffer (pH 6.4) and 20 ml distilled water.

Standard Solution (50 ml):

2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of standard drug (Diclofenac sodium) conc. of 10,50,100,200,300,400 and 500 µg/ml.

Test Solution (50 ml):

2 ml of Egg albumin, 28 ml of phosphate buffer and various concentrations of 10,50,100,200,300,400 and 500µg/ml. All of the above solutions were adjusted to pH6.4 using small amount of 1N HCL. The samples were incubated at 37˚C for 15 minutes and heated at 70˚C for 5 minutes. After cooling the absorbance of the above solutions was measured using UV-Visible spectrophotometer at 440nm and 241nm. The percentage inhibition of protein denaturation was calculated using the following formula[10]

$$\textit{Percentage Inhibition}=[(V_t/V_c)\times 1]\times 100$$

Where,

V_t=absorbance of test sample,

V_c=absorbance of control

- **In vitro Anticataract activity[11-12].**

1) Lens culture-

Fresh goat eyeballs were obtained from slaughterhouse was immediately transported to the laboratory at 0-4˚C. The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor (NaCl:140mM,KCL:5mM,MgCl:2mM,$NaHCO_3$:0.5mM,$NaH(PO_4)_2$:0.5mM,$CaCl_2$:0.4mM,and Glucose: 5.5mM) at room temperature and PH-7.8 for 72 hours.Penicillin G 32% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. At high concentrations,glucose in the lens was metabolized through sorbitol pathway and accumulation of polyols causing over hydration and oxidative stress. This lead to cataractogenesis.

2) Induction of in vitro cataract-

Glucose in a concentration of 55mM was used to induce cataract at high concentrations,glucose in the lens metabolizes through sorbitol pathway and accumulation of polyols (sugar alcohols)causing over hydration and oxidative stress. This lead to cataractogenesis. The lenses were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM served as normal control and 55 mM served toxic control) for 72hours.

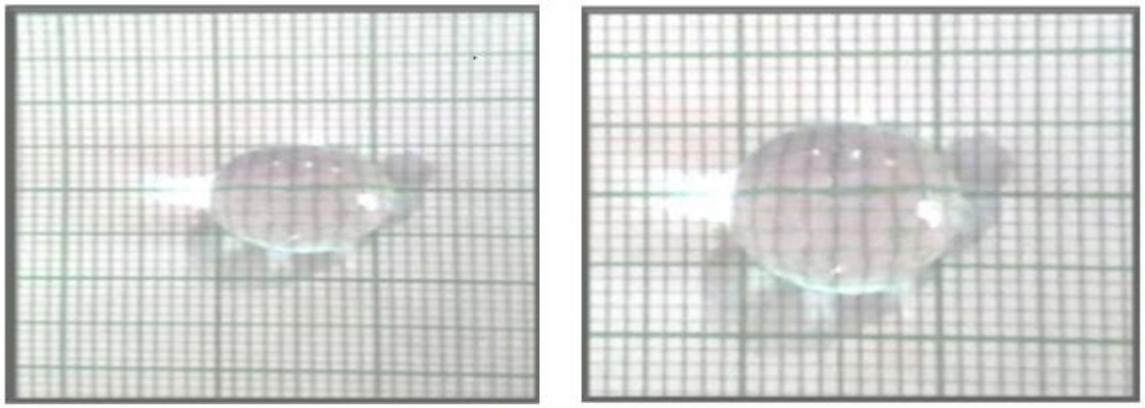
3) Study Design and Group-

Group 1: Aq.humor + Normal lens + glucose 5.5 Mm (Negative control A)
Group 2:Aq.humor + Normal lens + glucose 55 mm (Negative control B)
Group 3:Aq.humor + Normal lens + glucose 55 mm + 20µ g/ml Diosgenin
Group 4:Aq.humor + Normal lens + glucose 55 mm + 20µ g/ml std ascorbic acid .

4)Photographic Evaluation of Lens Opacity-

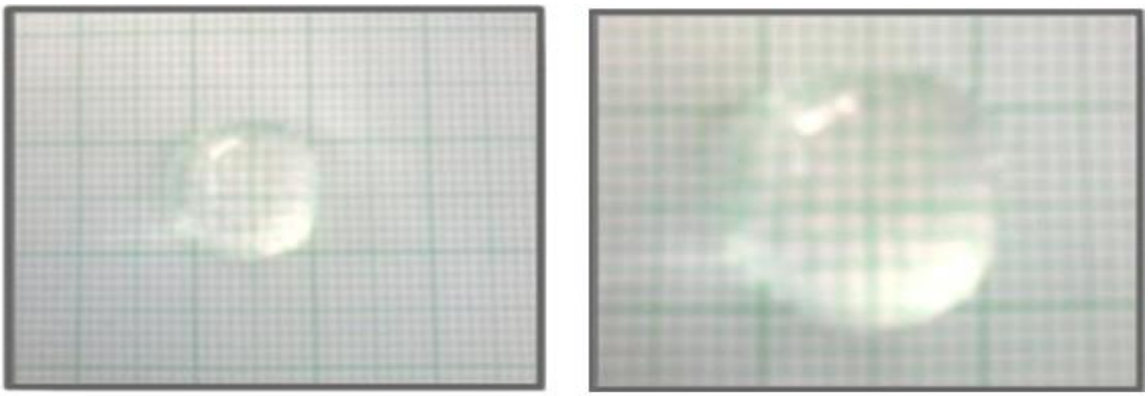
After 72 hours of incubation, lenses were placed on a wired mesh with posterior surface touching the mesh and the pattern of the mesh (number of squares clearly visible through the lens)was observed through the lens as a measure of lens opacity.

Photographic evaluation of anticataract activity



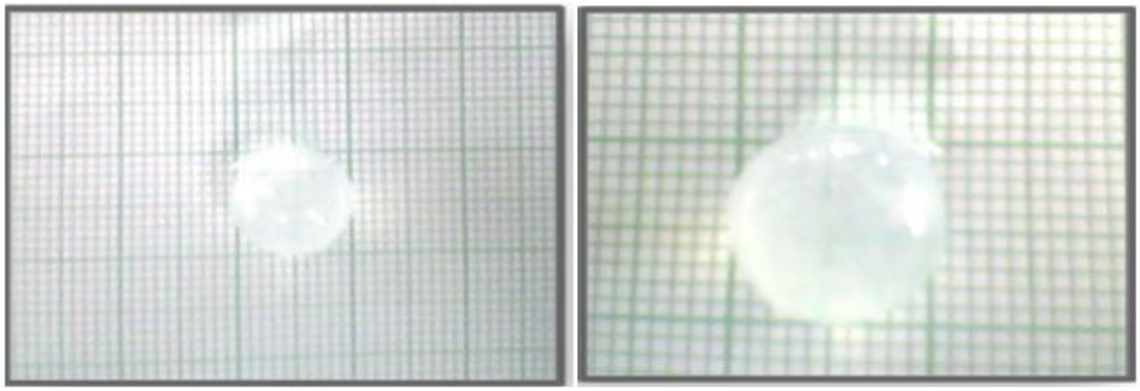
Normal View Zoom View

Fig 1 : Aqueous Humor (Normal Control)



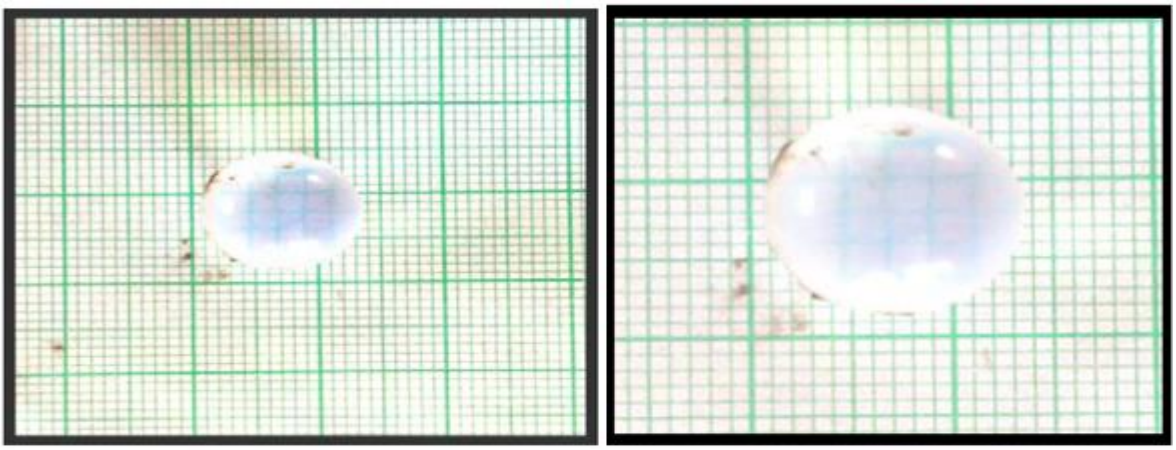
Normal View Zoom View

Fig 2a: Aqueous humor + 5.5 mM glucose (Negative Control)



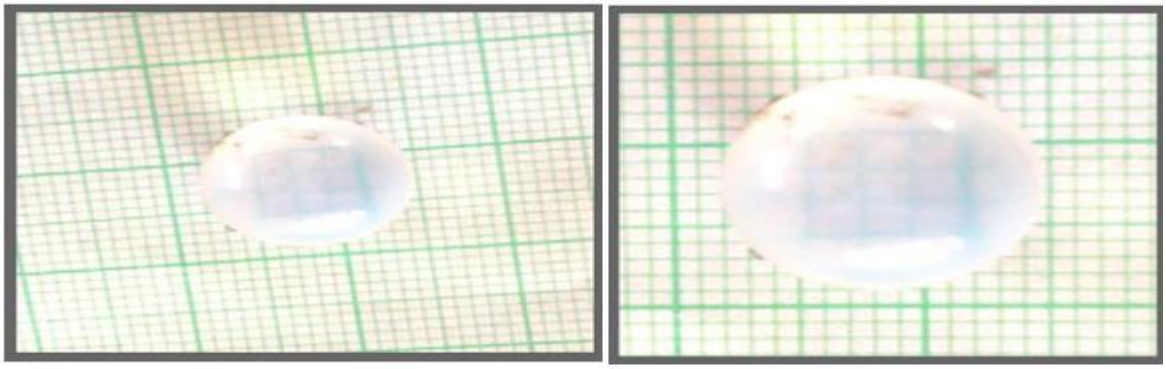
Normal View Zoom View

Fig 2b: Aqueous humor + 55 mM glucose (Negative Control)



Normal View Zoom View

Fig 3: Aqueous humor + 55 mM glucose +20 µg/ml Test compound (Diosgenin)



Normal View Zoom View

Fig 4: Aqueous humor +55 mM glucose +20µg/ml std. ascorbic acid(Positive Control)

The degree of opacity was graded as follows:
"0"-absence of opacity
"1"-slight degree of opacity
"2"-presence of diffuse opacity
"3"-presence of extensive thick opacity

RESULT

The percentage inhibition of protein denaturation by Diosgenin was depicted in table 1.

GROUP	CONC.(µg/ml)	%INHIBITION
DIOSGENIN	10	02.08%
	50	09.00%
	100	20.01%
	200	39.00%
	300	61.43%
	400	83.15%
	500	98.57%
DICLOFENAC SODIUM	100	96.53%

The results of the study showed that the diosgenin possess significant anti denaturing and anti-cataract activity.

Photographs of lenses in normal and experimental groups incubated with glucose are shown in fig 1-4.

Fig 1)shows the normal lens incubated with artificial aqueous humor showing complete transparency compared with the experimental groups. Fig 2a) is the lens incubated with glucose (5.5mM) for a period of 72 hrs showing clear lens because of low conc. of glucose does not show effect on lens. Fig 2b) is the lens incubated with glucose (55mM) for a period of 72 hrs showing complete opacification of lens due to high conc. of glucose. Fig 3 & 4)are the lenses incubated simultaneously with glucose (55mM) and diosgenin at a concentration of 20 µg/ml and std ascorbic acid at a concentration of 20µ g/ml respectively,showing a decrease in opacity compared to cataractous lenses and thus showing almost normal transparency in the lens.

Degree of opacity shown by compound :

Table 2

COMPOUND	DEGREE OF OPACITY
Positive Control	0
Negative Control(2a)	1
Negative Control (2b)	3
Test Compound(Diosgenin)	0
Standard Compound(Ascorbic Acid)	0

Table no 2 shows degree of opacity shown by diosgenin
Normal control: zero degree opacity is occurred,clear lens is obtained.
Negative control (a): slight degree of opacity is occurred, not found clear lens.
Negative control (b): presence of extensive thick opacity, because of high conc. of glucose inducecataractogenesis.
Test (diosgenin 20 g/ml): zero degree of opacity is occurred, clear lens is obtained. Test drug inhibit cataractogenesis.
Std (Ascorbic Acid 20 g/ml): Lens shows very slight degree of opacity and found as clear lens.

DISCUSSION

Denaturation of proteins is one of the well documented cause of cataract , also prolonged exposure to elevated glucose causes both acute reversible changes in cellular metabolism and long-term irreversible changes in stable micromolecules.
In vitro model for inducing cataract using glucose 55 mM provides an effective model on isolated lenses of goat. Incubation of the goat lenses in the media containing high glucose (55mM) conc. induce cataract has shown to cause considerable drop in Na^+/K^+ ATPase activity,with progression of opacity. The impairement of Na^+/K^+ ATPase cause accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibres leading to cataractogenesis. This alteration in the Na^+,K^+ ratio changes the protein content of the lens,leading to decrease in total proteins and oxidative stress causing denaturation of proteins leading to cause lens opacification.

CONCLUSION

To conclude, the study suggested that the Diosgenin possess antidenaturing and anticataract activities, which might be helpful in preventing or slowing the progress of cataract.

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